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REGULATION OF MUSCLE PROTEIN TURNOVER IN THE TUMOUR - BEARING HOST

by

ALICIA BEATRIZ STRELKOV

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

Animal Biochemistry

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FALL 1988

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Supervisor

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Date... Oct. 2 ... 1988 ...

Abstract

Two different tumours were compared in rats: Morris Hepatoma 7777 (MH) a slow growing non-metastasizing solid tumour , and Yoshida Acites Hepatoma (YAH) AH130 a metastasizing and rapidly growing ascites tumour and the role of PGE_2 in muscle protein catabolism was evaluated. Tumour bearing rats were compared to ad libitum and pair fed controls. Rats were also treated with naproxen (N), an inhibitor of prostaglandin synthesis.

Both tumours caused progressive anorexia and weight loss. For these and other parameters measured, the effects of YAH were quantitatively more severe than those of MH. Both tumours resulted in decreased weight and/or protein content of the soleus, extensor digitorium longus and epitrochlearis muscle; however, the extent of this wasting varied with muscle and tumour type. Muscle wasting induced by the tumours appeared to result both from increased protein degradation and decreased protein synthesis.

In all experiments, the effect of tumour implantation was greater than that of pair feeding, although restricted feed intake caused similar effects. The contribution of reduced food intake to the observed muscle wasting varied with tumour type. In the YAH reduced food intake was not a significant component; however in MH, decreased food intake accounted for approximately 40% of the muscle wasting seen.

YAH produced large amounts of PGE_2. Treatment of

rats bearing YAH with naproxen inhibited the increased muscle protein degradation and muscle protein loss (by 40%). Naproxen had no effect on muscle weight or protein degradation in rats bearing MH. These results would appear to implicate PGE_2 in the development of cachexia in the laboratory rat.

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INTRODUCTION

The objective of this chapter is to outline present knowledge of a) the regulation of muscle protein synthesis and degradation, b) alterations in protein metabolism in the tumour bearing-host and c) to discuss the possible mechanisms of tumour-induced muscle wasting. The review leads to the rationale and hypothesis of the thesis research.

1. Regulation of Protein Turnover in Skeletal Muscle

Whether a tissue grows or atrophies depends on the balance between the rates of protein synthesis and degradation. These processes occur continuously, and are collectively referred to as protein turnover (Fig.I-1). All proteins and tissues have characteristic rates of turnover, and are in dynamic equilibrium with the free amino acid pool. In Fig. I-2, typical proportions of the daily protein turnover in the rat attributed to different tissues are shown (Garlick et al. 1980). Similar quantitative information is presently not available for other species. Together, muscle and liver make up 50% of the daily turnover, skin and the gastrointestinal tract comprise almost 40%, and the remainder is accounted for by bone (8%), kidney (2.2%), spleen (1.8%), lung (1.1%), and brain (0.1%). Although its turnover rate is low (17%/day), the contribution of muscle is large due to its relative abundance (Waterlow, 1984). Liver, skin and

digestive tract, although smaller tissues, have higher turnover rates (60-90%/day) (Garlick 1980; Waterlow 1984), and make a large contribution to the daily turnover.

In skeletal muscle, as in all tissues, protein synthesis and degradation are precisely regulated, and vary under different physiological and pathological conditions. This regulation is an area of rapidly expanding research efforts (Baracos et al. 1984a; Goldberg et al. 1980; Tischler 1981; Beisel 1984; Goldberg 1976; Kameyama et al. 1979).

a. Circulating Hormones and other Factors

Various hormones, including insulin, thyroid hormones, growth hormone, glucocorticoids and the gonadal steroids have been shown to affect the rates of protein synthesis and degradation in muscle (Odedra et al. 1983; McGrath et al. 1982; Tischler 1981; Smith et al.1983; Buttery 1983; Stirewalt et al. 1983; Carter et al. 1982) (Table I-1). For example, insulin has an anabolic effect on skeletal muscle, stimulating amino acid and glucose transport, increasing protein synthesis and simultaneously decreasing protein degradation (Tischler 1981). Therefore, the changes in insulin concentrations and the sensitivity to insulin at the level of the receptor will affect muscle growth (Goldberg et al. 1980; Tischler 1981). In addition, rates of muscle protein synthesis and degradation depend upon food intake, the supply of branched chain amino acids to the muscle, and the level of contractile activity (Goldberg et al. 1980; Snell 1980; Tischler et al. 1982;

Lundholm et al 1982; Laurent et al. 1978; Goldspink 1978; Palmer et al. 1983; Rodemann et al. 1982b; Lewis et al. 1982; Palmer et al. 1985; Millward et al. 1985; Fulks et al.1975).

b. Prostaglandins

Prostaglandins (PG) are derivatives of 20 carbon fatty acids such as arachidonic acid which have a variety of regulatory functions in normal physiology, as well as a number of pathological processes including inflammation, regulation of immune response and fever (Dinarello et al. 1982a,b; Kuehl et al. 1980; Plescia et al. 1975; McKinley et al. 1986; Goodwin et al. 1983). Prostaglandins are neither stored nor concentrated in the cell, but are synthesized from fatty acids which are esterified in membrane phospholipids. For example, phospholipase A_2 causes the release of free arachidonic acid, this can be caused by a great variety of stimuli (eg. hormones, tissue injury, immunological reactions, cell injury, endotoxin, mechanical agitation, (Goodwin et al. 1983; Kuehl et al. 1980; Irvine 1982)); free arachidonic acid may then react with cyclooxygenase, the first enzyme leading to synthesis of prostaglandins and thromboxanes, or with lipoxygenase, the enzyme leading to leukotriene and lipoxin synthesis (Fig.I-3) (Gibson 1977; Samuelsson et al. 1987). After this step arachidonic acid is converted into different biologically active metabolites which include the different kinds of prostaglandins (eg. PGE_2, $PGF_{2\alpha}$, PGD_2), thromboxanes (TXA_2, TXB_2), leukotrienes (LTA_1, LTA_2, LTB_4) and lipoxins

(LXA, LXB). The spectrum of metabolites produced is determined by the enzyme content of the tissue under consideration (Goodwin et al. 1983; Kuehl et al. 1980; Gibson 1977; Markelonis et al.1975). Aspirin and related compounds such as indomethacin and naproxen, inhibit prostaglandin synthesis by inactivation of the cyclooxygenase branch of the pathway.

Until recently, the possibility that prostaglandins might be important in the regulation of muscle protein turnover in normal or pathological conditions had not been considered. Goldberg and coworkers (Rodemann et al. 1982a) showed that when arachidonic acid was added to skeletal muscle incubated in vitro, protein synthesis and degradation were stimulated. Exogenously added $PGF_{2\alpha}$ mimicked the effect of arachidonic acid in stimulating protein synthesis. Similarly, exogenously added PGE_2 stimulated protein degradation in incubated muscles (Rodemann et al. 1982a; Baracos et al. 1984b; Goldberg et al.1984). Recent work by Reeds and coworkers suggest that alterations in muscle protein synthesis induced by insulin, mechanical stretching and glucocorticoids are also mediated by $PGF_{2\alpha}$. For example, they found that mechanical stretching of incubated skeletal muscle increased the release of $PGF_{2\alpha}$ and other prostaglandins including PGE_2, and stimulated protein synthesis. The addition of indomethacin, a cyclooxygenase inhibitor, prevented the increase in protein synthesis. Exogenously added $PGF_{2\alpha}$ mimicked the effect of mechanical stretching on protein synthesis (Smith et al. 1983; Palmer et al. 1983). Insulin increased protein synthesis in

skeletal muscle, also by increasing the concentration of $PGF_{2\alpha}$. This effect was inhibited by indomethacin (Reeds et al. 1983; Reeds et al. 1985). The hypertrophy which follows training after disuse atrophy also appears to be mediated by $PGF_{2\alpha}$ (Templeton 1986). Finally, Reeds and coworkers found that the synthetic glucocorticoid, dexamethasone, decreased protein synthesis and the concentrations of $PGF_{2\alpha}$ in incubated muscles, possibly by inhibiting the activity of phospholipase A_2 (Reeds et al. 1984). Thus in vitro experiments demonstrated that PG influence the rates of synthesis and degradation of muscle proteins. Whether prostaglandins affect turnover in other tissues or whether prostaglandins affect other aspects of protein metabolism remain to be fully explored. A great number of inflammatory events, either systemic or local, involve both increased prostaglandin production and alterations in muscle protein turnover. Although it seems possible that in a variety of pathological states such as infection, inflammation and injury; prostaglandins may be involved in the regulation of turnover in muscle or other tissues, this question has only began to be examined.

c. Factors Derived from Mononuclear Phagocytes.

Mononuclear phagocytes are a major cellular component of the reticuloendothelial system and include a circulating form (monocytes) and a fixed tissue form (macrophages). Mononuclear phagocytes are central in host defense responses, and exhibit anti-tumour, anti-bacterial and inflammatory activity (Adams &

Hamilton, 1984). Mononuclear phagocytes synthesize and release more than 75 defined molecules (Table I-2), including hydrolytic enzymes, metabolites of arachidonic acid, reactive oxygen species, complement components and cytokines (cellular growth factors), which collectively mediate host defense. In addition to being a major source of prostaglandins, and particularly PGE_2, mononuclear phagocytes have recently been shown to produce other factors affecting protein turnover in muscle, particularly interleukin-1 (See below).

Interleukin-1 is a 15 kd peptide produced by mononuclear phagocytes and other cell types. It acts like a hormone, mediating host responses to inflammation, infection and immunologic reactions (Loda et al. 1984; Dinarello, 1985; Dinarello 1984b; Kampschmidt 1984; Dinarello et al. 1982a; Durum et al. 1985). Its activities include induction of fever in by its action on the hypothalamus, activation and proliferation of lymphocytes and neutrophils, initiation of the synthesis of the acute phase proteins by the liver, and stimulation of PGE_2 production in a large number of tissues. Interleukin-1 acts on bone marrow to induce neutrophilia, and it also appears to initiate systemic metabolic alterations associated with infection, such as the net breakdown of skeletal muscle, and trace metal redistribution (Table I-3) (Baracos et al. 1983; Dinarello 1984b; Kampschmidt 1984; Goldberg et al. 1984; Fagan et al. 1987). The production of interleukin-1 is stimulated by bacterial products, some immunological reactions and inflammatory agents (Cannon et al. 1986). When interleukin-1 -

containing fractions from human peripheral blood monocytes were added to isolated rat muscle, protein degradation increased by 60 to 120%, while protein synthesis was not affected (Baracos et al. 1983). The increased proteolysis was accompanied by a three to ten-fold increase in the rate of release of PGE_2 by the muscles. PGE_2 mediated the protein catabolism induced by interleukin-1, since indomethacin abolished both the increased PGE_2 production and protein breakdown. These effects were observed when muscles were incubated with interleukin-1 at 37^oC, and thus occur independently of the rise in body temperature (fever) induced by interleukin-1. In related studies it was found that increased temperature of incubation also increased muscle protein degradation, and that the effects of interleukin-1 and increased temperature of incubation (39^oC) were additive in promoting muscle protein breakdown (Baracos et al.,1983). These results suggest that activated mononuclear phagocytes may produce factors which activate the catabolism of protein, coordinately with primary host defense functions.

The results summarized above suggest that prostaglandins may be important in regulation of muscle protein turnover *in vivo*. The prostaglandins may be produced locally in muscle in response to a variety of mediators or may arrive via the systemic circulation. Several studies support the suggestion that the rapid onset of muscle wasting that takes place during fever or sepsis in vivo is signalled by a prostaglandin dependant mechanism. For example, systemic infections induced

by injection of *Streptococcus pneumoniae* in the rat promoted rapid muscle protein loss, and reduced contractile function, as measured by peak twitch and tetanic tension (Ruff and Secrist 1984). These effects were inhibited by the in vivo administration of indomethacin. Injections of live *Escherichia coli* into rats caused fever, and skeletal muscles from these rats showed increased proteolysis and PGE_2 production (Fagan et al. 1985). When the rats were pre-treated with indomethacin the increases in protein degradation and prostaglandin production were inhibited (Fagan et al. 1985).

In addition, Clowes and coworkers have isolated a factor from plasma of septic patients which promotes protein degradation in rat muscle, as does interleukin-1 (Clowes et al. 1983). This 4.2 kd "proteolysis inducing factor" may be a circulating fragment of interleukin-1 (Dinarello et al. 1984), which causes lymphocyte activation, acute phase protein synthesis and muscle proteolysis, but which does not induce fever (Clowes et al. 1983; Dinarello et al. 1984). Solutions of purified interleukin-1 when cold stored or treated with trypsin had the 4.2 kd proteolysis inducing factor fragment. Both proteolysis inducing factors and interleukin-1 have been isolated from blood and pleural fluid from patients with abcesses (Clowes et al. 1985). However, it is very difficult to show interleukin-1 in plasma and the 4.2 kd piece may be a major circulating form. The relative proportions of the 15 kd and 4.2 kd forms are not known and the subsequent metabolism and/or clearance of the proteolysis inducing factor remain to be

clarified (Clowes et al. 1985; Clowes et al. 1983; Dinarello et al. 1984a).

2. Protein Metabolism in the Tumour - Bearing Host.

Prominent muscle wasting is characteristic of various pathological states. In particular, patients with fever, sepsis, traumatic injury, surgery or burns show a marked loss of body weight and protein (Beisel 1984). In adults, these conditions lead to negative nitrogen balance that is larger than that seen during fasting. In rapidly growing young there is typically an impairment of growth. Whole body wasting and protein loss from skeletal muscle are also prominent clinical signs in the tumour-bearing host (Preston et al. 1987; Theologides 1972; Jeevanandam et al. 1984). The frequency with which extensive wasting occurs in different cancer patients with different types of tumours varies from 16 to 84% (Oliff et al. 1987; Strain 1979). The size of response of skeletal muscle wasting varies with different types of tumours and is proportional to the duration of the illness. For example, in a recent study, tumour-bearing patients that had lost 30% of their pre-illness body weight suffered a 74% loss of their skeletal muscle (Preston et al. 1987). In addition to progressive losses of weight and muscle protein, many cancer patients also experience anorexia, nausea, asthenia, weakness and anemia. This collective of symptoms is known as cancer - associated cachexia. The progressive wasting characteristic of the

syndrome contributes directly to cancer mortality, and may also reduce the ability of patients to tolerate agressive chemotherapy or radiotherapy regimes. The prevalence of cancer - associated cachexia and its impact on mortality constitutes a significant problem in medical oncology. However, the underlying basis of this metabolic response remains to be adequately explained.

a. Human studies

In order to establish how changes in protein turnover account for wasting seen in cancer patients, attempts have been made to determine whole body and muscle protein turnover in cachectic patients with a variety of different malignant tumours including carcinomas, adenocarcinomas, lymphomas and leukemias (Table 1-4). Relatively little information exists on the rate of whole body protein turnover in tumour-bearing patients. Kien et al. (1983) measured whole body protein turnover in children with leukemia-lymphoma, showing weight loss and fever, compared to healthy children. Using the method of Waterlow et al. (1978) employing a single dose of ^{15}N glycine, they concluded that rates of protein synthesis increased (50%) and rates of protein degradation also increased in the tumour bearing children.

Studies of whole body turnover, while having the advantage of being non-invasive, average together the component tissue rates of protein synthesis and degradation, making it impossible to distinguish tissue-specific effects. Therefore, a number of investigators have used alternative approaches to estimate

muscle protein synthesis and degradation. For example, Lundholm et al. (1976) showed decreased rates of protein synthesis and increased activity of a proteolytic enzyme, Cathepsin D, in biopsied skeletal muscle incubated in vitro from patients with different types of malignant tumours. Emery and coworkers (1984a), after constant i.v. infusion of ^{13}C leucine took biopsies of quadriceps muscle (Rennie et al. 1982a; Halliday 1981) to measure in vivo muscle protein synthesis. They found that protein synthesis in skeletal muscle was reduced by more than 80% in cachectic cancer patients compared to healthy controls. Another approach has been to measure net protein balance across the hind limb, by measuring the arterio-venous differences of amino acids in the femoral circulation. Since the hind limb is composed in large part of skeletal muscle, this determination is taken as an index of muscle protein balance. Benegard and coworkers (1982), using this approach observed a decrease (32%) in net tyrosine release across the leg in cachectic patients with different carcinomas compared to healthy controls. Lundholm et.al. (1982), estimated actomyosin protein degradation by the release of 3-methylhistidine and tyrosine across the femoral circulation of cachectic cancer patients with different carcinomas. They found that the healthy controls had a significantly higher 3-methylhistidine and tyrosine release than the cancer patients. These results led the authors to suggest that the skeletal muscle wasting may be caused by decreased protein synthesis, because there was no apparent increase of these indices of net protein degradation. Thus

there is general agreement within the human studies, that muscle protein synthesis is depressed in tumour - bearing individuals. A variety of indirect measures of muscle protein breakdown suggest that degradative rates either rise or stay the same.

b. Experimental Animal Studies.

Because of the ethical limitations of studies of protein metabolism in human cancer patients, studies have also been carried out on experimental animal species, with a wide range of experimentally induced tumours, including methylcholanthrene - induced sarcoma, Erlich ascites tumour, Walker carcinoma 256, Yoshida ascites hepatoma AH130, and fibrosarcoma. These studies are summarized in Table I-4. In these studies, much attention has been devoted to skeletal muscle, although hepatic and cardiac protein synthesis have also been measured. In general, muscle protein synthesis is depressed in tumour bearing rats and mice, and muscle protein degradation is increased. Lundholm and coworkers (1981), measured the incorporation rate of ^{3}H and ^{14}C leucine into myofibrillar and sarcoplasmic protein of mice implanted with a methylcholanthrene-induced sarcoma. Tumour-bearing mice had decreased protein synthesis (40-50%) in skeletal muscle. These authors also found increased cathepsin D activity in skeletal muscle, suggesting that the tumour caused increased proteolysis in skeletal muscle. Kawamura et al.(1982) found decreased protein synthesis in skeletal muscle (25-40%) in rats with advanced RNC-254 fibrosarcoma growth compared to healthy controls, using a constant infusion of ^{14}C tyrosine.

Pain and coworkers (1984), measured the rate of protein synthesis in mice injected with Erlich ascites tumour, using the flooding dose of ^{3}H phenylalanine method developed by their group (McNurlan et al. 1979; Garlick et al. 1980). They found decreased rates of protein synthesis (-44%) in gastrocnemius muscle compared to healty controls. Norton et al.(1981), studied protein metabolism using a constant infusion of ^{15}N glycine in rats with methylcholathrene-induced sarcomas. Urine was analyzed for ^{15}N urea for determining whole body protein turnover which was increased in tumour rats, and tissue ^{15}N enrichment to determine tissue fractional synthetic rate determined by the method of Stein et al. (1976; 1977) which was found to be decreased by 45% in gastrocnemius muscle of tumour - bearing animals. These authors also measured urinary 3 - methylhistidine as an index of myofibrillar protein catabolism; this was increased (18%) in tumour bearing rats compared to healthy controls. In a study by Goodlad et al. (1981), rats bearing Walker carcinoma were used. Two methods were used to determine protein degradation, for this, soleus and extensor digitorum longus muscles were dissected and incubated in vitro with ^{14}C leucine to determine $^{14}CO_2$ production and tyrosine release into the incubation medium. Tumour bearing rats had increased protein breakdown in these muscles, however the soleus was less affected than the extensor digitorum longus. Tayek et. al. 1986, measured protein synthesis with a single dose of ^{14}C leucine in rats implanted with Walker carcinoma, the rats bearing large tumours had decreased protein

synthesis (33%). Tessitore et al. (1987) measured in vivo protein synthesis and degradation using ^{14}C bicarbonate in rats bearing Yoshida ascites hepatoma AH-130. The tumour-bearing rats showed an increase in protein degradation in gastrocnemius muscle and no change in protein synthesis. Emery and coworkers (1984b) measured in vivo protein synthesis in quadriceps and gastrocnemius muscle of mice, and found that protein synthesis was decreased by 58% compared to healthy controls using a constant infusion of ^{14}C tyrosine.

Thus, using a considerable variety of methods and types of experimental tumours, it appears that muscle protein synthesis and degradation change in opposite directions, contributing to net protein loss.

3. Mechanisms of Muscle Protein Wasting in the Tumour-Bearing Host.

The loss of skeletal muscle seen in tumour bearing individuals may be due to decreased protein synthesis, increased protein degradation or a combination of both. As summarized above, it appears that both modifications in synthetic and catabolic rates contribute to this loss. Many factors affect tissue rates of protein synthesis and degradation, and it appears unlikely that muscle wasting associated with progressive tumour growth is related to a single regulatory factor. Current literature in the area covers a wide range of possible factors. An important factor is the influence of reduced dietary intake

and/or the high nutrient sequestering ability of the tumour, to generate a malnourished state. Alternatively, specific hormonal signals may be released either as a component of the host defense response or by a tumour, to directly induce muscle protein catabolism (Fig. I-4).

a. The Malnourished State

i) Tumour-induced anorexia

Anorexia (leading to a low food intake) is a symptom frequently present in the tumour bearing host (DeWys 1979). A variety of results suggest that cachectic cancer patients show metabolic peripheral changes quantitively similar to those suffering from nutrient deprivation, suggesting that tumour-host metabolism is not unique, but may represent a normal physiological response to nutritional deprivation caused by reduced food intake (Lundholm et al. 1981; Benegard et al. 1982; Karlberg et al.1981). The normal metabolic response to reduced food intake, is supression of muscle protein synthesis due to the lack of substrate supply (Stein 1982). Muscle protein degradation may be increased in the short term but later protein-sparing mechanisms come into play (Millward et al. 1976). Insulin appears to be primary in influencing these changes. Insulin has an anabolic effect on skeletal muscle, stimulating protein synthesis and inhibiting protein degradation. Circulating levels of insulin fall with decreased food intake, leading to a net release of amino acids from skeletal muscle (Goldberg et al. 1980; Tischler 1981). In a

recent study insulin levels were found to be low in tumour bearing animals and in animals after prolonged starvation (Devereux et al. 1985). Exogenously administered insulin during nutritional support in tumour bearers significantly increased protein synthesis and decreased protein catabolism in skeletal muscle (Burt et al.1984). However, others showed that the changes in muscle protein metabolism cannot be acounted for solely by the reduction in food intake (Karlberg et al. 1981). Emery et al. (1984a,b) demonstrated that fasting healthy humans for 18 h decreased protein synthesis by 50%, but that cancer patients fed 70% of the intake of normal controls reduced protein synthesis of the whole body by approximately 80%. In mice, that were fed half of their normal food intake, showed that muscle protein synthesis was affected to a lesser extent than tumour bearing mice with only a 15% decrease in food intake (Emery et al.1984a,b). In order to establish the proportion of wasting due to decreased intake, studies with experimental animals are frequently done with pair fed controls, where a control group receives the same food intake as the tumour bearing individuals, and a third group that receives ad lib feed intake (Lundholm et al. 1981; Kawamura et al. 1982; Goodlad et al. 1981; Tayek 1986). In human studies, malnourished non cancer patients, and well nourished control patients are used as controls (Lundholm et al. 1982; Benegard et al. 1982). Although a large proportion of the catabolism seen in these studies appears to be due to the decreased food intake, there is an additional effect of the tumour that cannot be accounted for by

the decreased intake.

ii) Substrate sequestration by the tumour

In addition a to decreased dietary supply of nutrients, the tumour sequesters large amounts of glucose, amino acids and other nutrients to grow, competing with other tissues for available substrates. This contributes to a chronic state of malnutrition of the host (Landel et al. 1982; Lundholm et al. 1981; Stein 1982). Although regulation of substrate sequestration and utilization by different tumours has not been characterized in detail, the basis for active substrate accumulation by tumours has been the subject of some investigations. For example, one hypothesis concerns the altered regulation of pyruvate dehydrogenase (PDH) in tumour cells (Fields et al. 1982). PDH is a key enzyme in the regulation of carbohydrate and fat metabolism, preventing the loss of lean body mass during fasting. With low food intake fat oxidation normally increases, leading to an increase in acetyl CoA concentrations supplying ATP for energy. This increase in acetyl CoA and ATP concentrations leads to the inactivation of PDH reducing the loss of pyruvate and its precursors (amino acids from proteolysis). As pyruvate is a three carbon precursor for glucose and non-essential amino acids synthesis, once it is converted to acetyl CoA (two carbon compound), and enters the TCA cycle it is irreversibly lost as CO_2 and water accompanied by ATP synthesis (Fig. I-5). So if PDH was active in the tumour compared to the host, there would be increased protein degradation in skeletal muscle to supply amino acids for

the production of glucose which is used by the tumour (For more detailed information see Fields et al. 1982).

iii. Specific catabolic signals

By contrast to a substrate-sequestration hypothesis for tumour-induced tissue wasting, there may be specific hormonal signals for skeletal muscle to increase protein degradation; these may be generated by the host or the tumour. It should also be recognized that the two hypotheses are not mutually exclusive. For example, many patients bearing tumours show clinical features of hyperthyroidism with increased lipid, glucose and protein metabolism and elevated energy expenditure (DeWys 1982). Some evidence is available pertaining to the circulating levels of thyroid hormones in tumour - bearing individuals (Persson et al. 1985; DeWys 1982). A minimum level of thyroid hormones are essential for normal growth of muscle; T_3 is especially active in stimulating the metabolism of muscle cells. Hypothyroidism affects protein metabolism, depressing protein synthesis in skeletal muscle. At high concentrations thyroid hormone depresses protein synthesis and causes an even greater increase in protein degradation in skeletal muscle (Goldberg et al. 1980). The sensitivity of different tissues to these hormones has not yet been studied, and the literature remains inconclusive as to the role of these hormones in tumour - induced catabolism.

b. Hypothesis and Objectives of the Present Study

In addition to factors mentioned above, we propose that

interleukin-1 and/or prostaglandin E_2 may be directly responsible for the initiation of muscle protein catabolism in tumour-bearing hosts (Kien et al. 1983), as has been suggested in fever and infection. Several observations have already created considerable interest in the effects of prostaglandins in cancer patients (Levine, 1981; Karmali, 1980). A number of experimentally induced tumours in animals as well as those occuring naturally in man have been found to produce prostaglandins (PGE_2 in particular) (Narisawa et al. 1981; Plescica et al. 1975; Yoenda et al. 1985; Hirata et al. 1984; Husby et al. 1977; Bennet et al. 1977). Some examples include lymphoma, neuroblastoma, pheocromocytoma, islet cell tumours, colonic, breast, thyroid and renal cell carcinomas, mouse sarcoma and fibrosarcoma, and virus induced sarcomas (Levine, 1981). In some tumours PGE_2 causes bone resorption (Tashjian et al. 1974; Seyberth et al. 1977; Voelkel et al. 1975), immunosuppresion (Goodwin et al. 1983) and in vitro studies showed that PGE_2 can stimulate tumour cell migration (Young et al. 1986). In addition, increased PGE_2 levels are found in breast cancer with poor prognosis and $PGF_{2\alpha}$ is increased in tumours with good prognosis (Vergote et al. 1985). The use of cyclooxygenase inhibitors like indomethacin can inhibit mammary carcinogenesis (Karmali et al. 1986). These elevated levels of PGE_2 in tumour-bearing individuals could act directly on muscle to induce net breakdown.

Similarly, a number of transformed cells are known to produce interleukin-1. These include monocytic leukemias from

mouse (P388D1 & J774) and man (THP-1, U937), human melanomas, E-B virus- transformed human B lymphocytes, transformed mouse keratinocytes (PAM212), Hodgkin's tumour cells, histiocytic lymphoma and renal cell carcinomas (Dinarello, 1984; Durum et al. 1985; Dinarello et al. 1980; Dinarello et al. 1982b). Accordingly, fever accompanies various malignancies, including Hodgkin and non-Hodgkin's lymphomas, hepatomas, hypernephromas and atrial mixomas, (Dinarello, 1984; Durum et al. 1985). It is not clear whether these cells produce interleukin-1 spuriously, or the interleukin-1 produced plays a role in the pathogenesis of the tumour.

Interleukin-1 is cytotoxic to some tumour cells by working synergistically with interferon and interleukin-2 to increase tumour killing by natural killer cells (Dinarello 1985; Dempsey et al. 1982). Therefore, interleukin-1 is thought to participate in host defense against malignancy by stimulating the immune system.

A peptide called "Proteolysis inducing factor" (PIF), that appears to be an active fragment of interleukin-1 and that has been discovered in septic and traumatized patients, has been found in plasma of rats bearing fibrosarcomas, with the same proteolysis inducing factor activity as in septic, traumatized and fasted hosts (Clowes et al. 1983; Dinarello 1984b; Clowes et al. 1985). However it is not known if the proteolysis inducing factor and interleukin-1 are synthesized by the tumour cells or by the host in response to the tumour as part of the defense mechanism.

In our studies we propose to test the involvement of interleukin-1 and prostaglandins in muscle atrophy subsequent to the implantation of experimental tumours in the rat. The two experimental models to be employed are Morris Hepatoma 7777 (Morris 1965) and Yoshida ascites hepatoma AH 130 (Tessitore et al. 1987; Travisani et al. 1980; Mahmud et al. 1981). Both types of tumours induce severe wasting and muscle protein catabolism in their host. The Yoshida hepatoma is known to be a prostaglandin - producing tumour, while the Morris hepatoma is not known to produce PGE_2.

Three major areas of experimentation are involved:

1) To characterize alterations in muscle protein turnover, particularly in skeletal muscle, during progressive tumour development.

2) To probe the possible involvement of interleukin-1 and PGE_2 in these effects.

3) To test the therapeutic prediction that muscle protein loss in tumour-bearing animals may be prevented by inhibitors of prostaglandin synthesis.

Table I.1. Factors Affecting Protein Turnover in Skeletal Muscle.

Factor	Protein synthesis	Protein degradation	Overall
Growth hormone (somatomedin)	↑	↑	Anabolic
Insulin	↑	↓	Anabolic
Glucagon	↓	–	Catabolic
Thyroid Hormone			
normal levels	↑↑	↑	Anabolic
high levels	↑?	↑↑	Catabolic
Glucocorticoids	↓	↑	Catabolic
Gonadal Steroids (androgens, estrogens)	↑?	↓?	Anabolic
Contractile work, stretching	↑	↓	Anabolic
Branched chain amino acids	↑	↓	Anabolic
Prostaglandin E_2	–	↑	Catabolic
Prostaglandin $F_{2\alpha}$	↑	–	Anabolic
Interleukin-1	–	↑	Catabolic
Calcium		↑	Catabolic
Glutamine	↑	↓?	Anabolic

Table I.2. Secretory products of activated macrophages.

1.Lysosomal hydrolases

2.Lysozyme

3.Neutral proteases

-elastase

-collagenase

-plasminogen activator

-cytolytic protease

4.Arachidonic acid metabolites

PGI_2, TXB_2, PGE_2, PGF_2, LTC_4, LTB_4

5.Reactive Oxygen Metabolites

$O_2^{-\cdot}$, H_2O_2, $OH^{\cdot}$

6.Complement components

7.Cytokines (Cellular Growth Factors)

-interleukin-1

-interferon

-tumour necrosis factor

Table I.3. Multiple Biological Activities Attributed to Interleukin-1

Fever (in rabbits, mice, and rats)	Chemotaxis of
Hypoferremia	Monocytes
Hypozincemia	Neutrophils
Hypercupremia	Lymphocytes
Increased	Stimulation of PGE_2 in
Blood neutrophils	Hypothalamus
Hepatic acute-phase proteins	Cortex
Bone resorption	Skeletal muscle
Cartilage breakdown	Dermal fibroblasts
Muscle proteolysis	Synovial fibroblasts
Slow-wave sleep	Chondrocytes
Endothelial procoagulation	Macrophage/monocytes
Chondrocyte proteases	Endothelium PGI_2
Synovial collagenases	Decreased
Endothelial neutrophil adherance	Hepatic albumin synthesis
Neutrophil degranulation	Appetite
Neutrophil superoxide	Brain binding of opioids
Interferon production	Augmentation of
Proliferation of	T-cell receptors
Fibroblasts	B-cell receptors
Glial cells	NK activity
Mesangial cells	IL-2 production
Synovial fibroblasts	Lymphokine production
EBV B-cell lines	

Table I.4. Effects of tumours on muscle protein synthesis and degradation

Species	Tumour	Degradation	Synthesis	Ref.
Human	carcinomas	—	↓	Lundholm et al. 1982
Human	various	↑	↓	Lundholm et al. 1976
Human	carcinomas	↑	↓	Benegard et al. 1982
Human	carcinomas	—	↓	Emery 1984
Human	carcinoma	↑	↓	Burt et al. 1984
Human	Lymphoma	↑	↑	Kein et al. 1983
Mice	methylcholanthrene sarcoma		↓	Lundholm et al. 1981
Mice	Erlich ascites		↓	Pain and Garlick 1980
Mice	XK1	↓	↓	Emery et al. 1984b
Mice	Erlich ascitis		↓	Pain et al. 1984
Rat	methylcholanthrene sarcoma	↑	↓	Norton et al. 1981
Rat	Walker Carcinoma		↓	Tayek et al. 1986
Rat	Yoshida hepatoma	↑	—	Tessitore et al. 1987
Rat	Fibrosarcomas	↑	↓	Kawamura et al. 1982
Rat	Walker Carcinoma	↑		Goodlad et al. 1981

Fig. I-1: The process refered to as protein turnover is made up of 2 major constituent processes: the catabolism of cell protein and the de novo synthesis of proteins from free amino acid.

Figure 1.1 PROTEIN TURNOVER

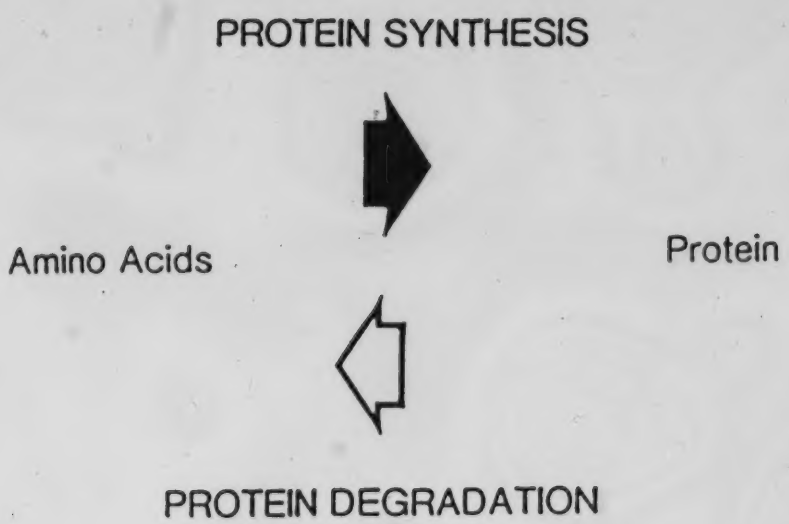


Fig. I-2: Protein synthesis rates for different tissues of laboratory rats from data obtained by the 'flooding dose' technique (Garlick et al. 1980) as summarized by Waterlow (1984).

Figure 1.2

PERCENT OF WHOLE BODY PROTEIN TURNOVER

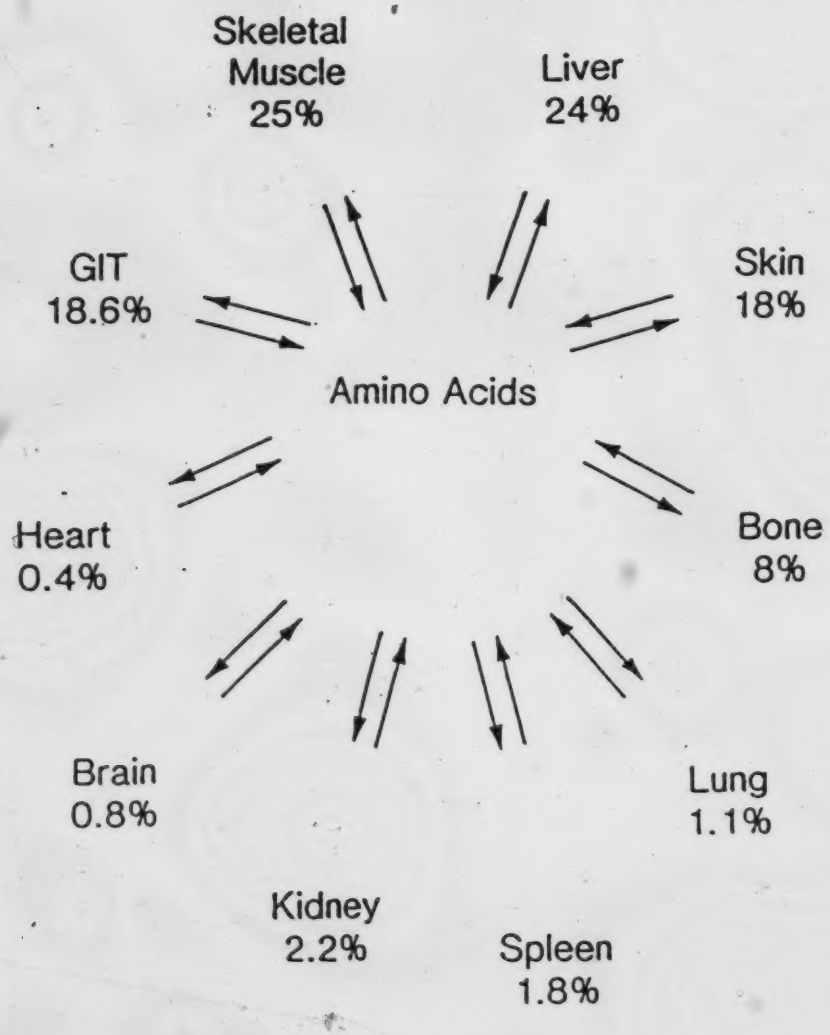


Fig. I-3: Diverse agonists an stimili result in activation of phohplipase A_2 and cleavage of arachidonic acid from sites of esterification into membrane phospholipids. Free arachidonate may be converted via the activity of cyclooxygenase to prostaglandins and thromboxanes, or by lipoxygenase to leukotrienes, hydroperoxy eicosatetraenoic acids (HPETE) and hydroxy eicosatetraenoic acids (HETES). *Aspirin like drugs inhibit cyclooxygenase.

Figure 1.3

Cascade of Arachidonic Acid Metabolism

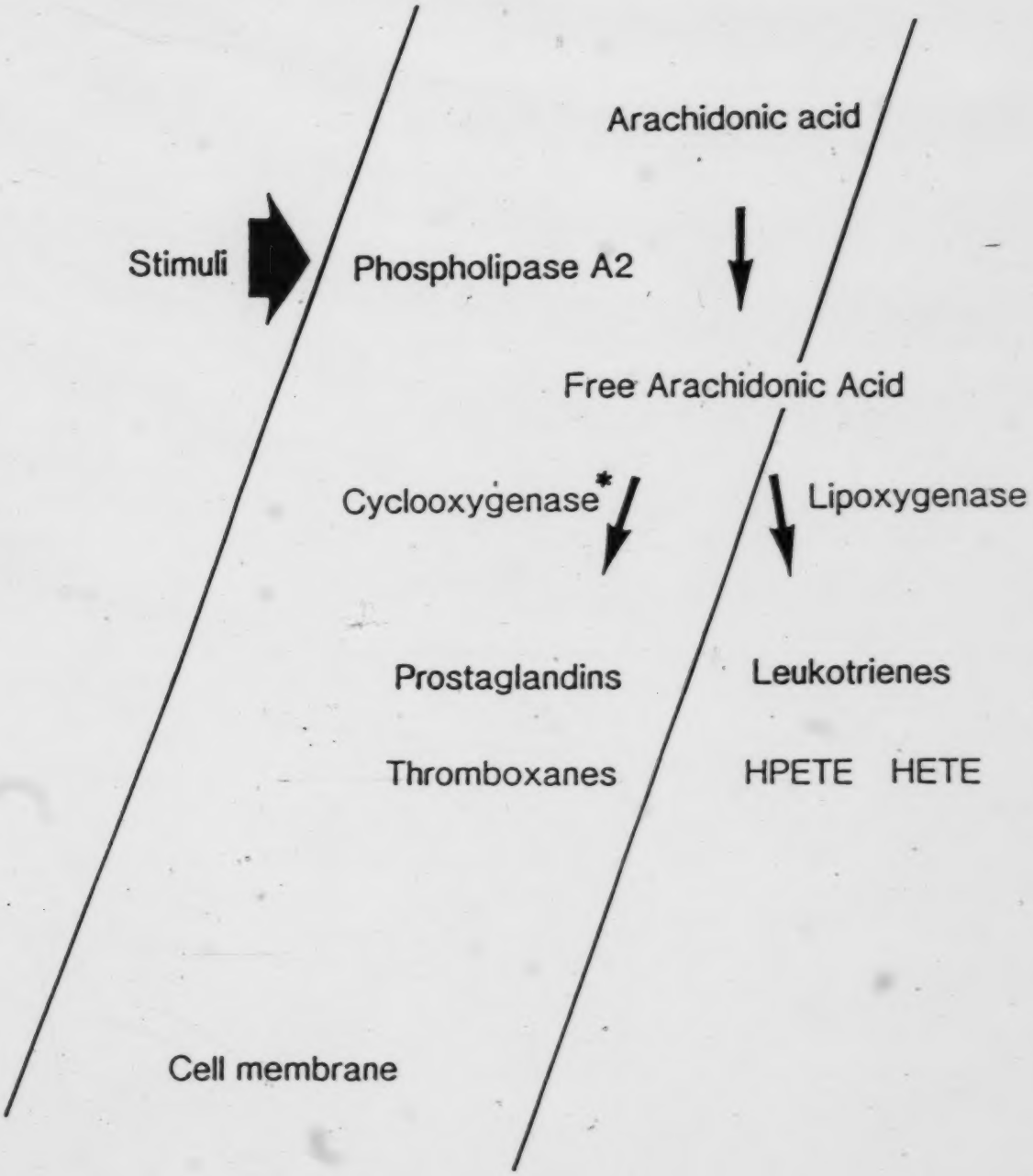


Fig. I-4: Reduced dietry intake supply of amino acids (1) and amino acid sequestration by the tumour (2) lead to increased muscle protein degradation (3) and decreased muscle protein synthesis (4). Net catabolism of other body tissue proteins also occurs (5). In addition, specific catabolic factors for muscle may originate from the tumour or the host organisms (-------->).

FIGURE I.4 MUSCLE PROTEIN CATABOLISM IN THE TUMOUR - BEARING HOST

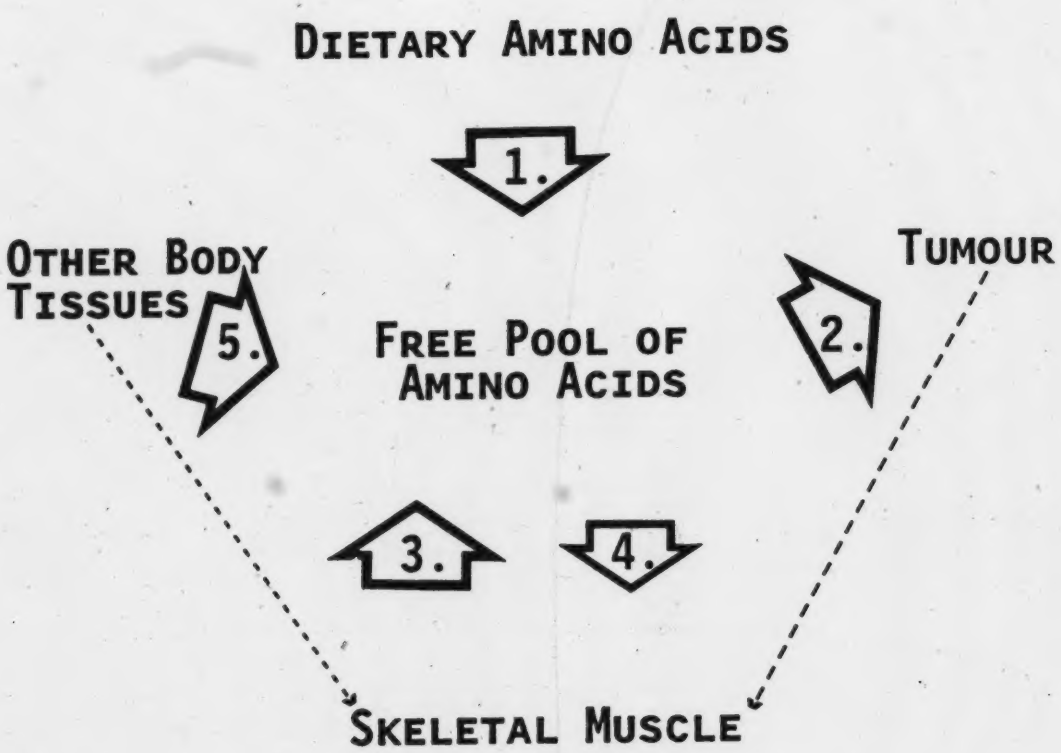
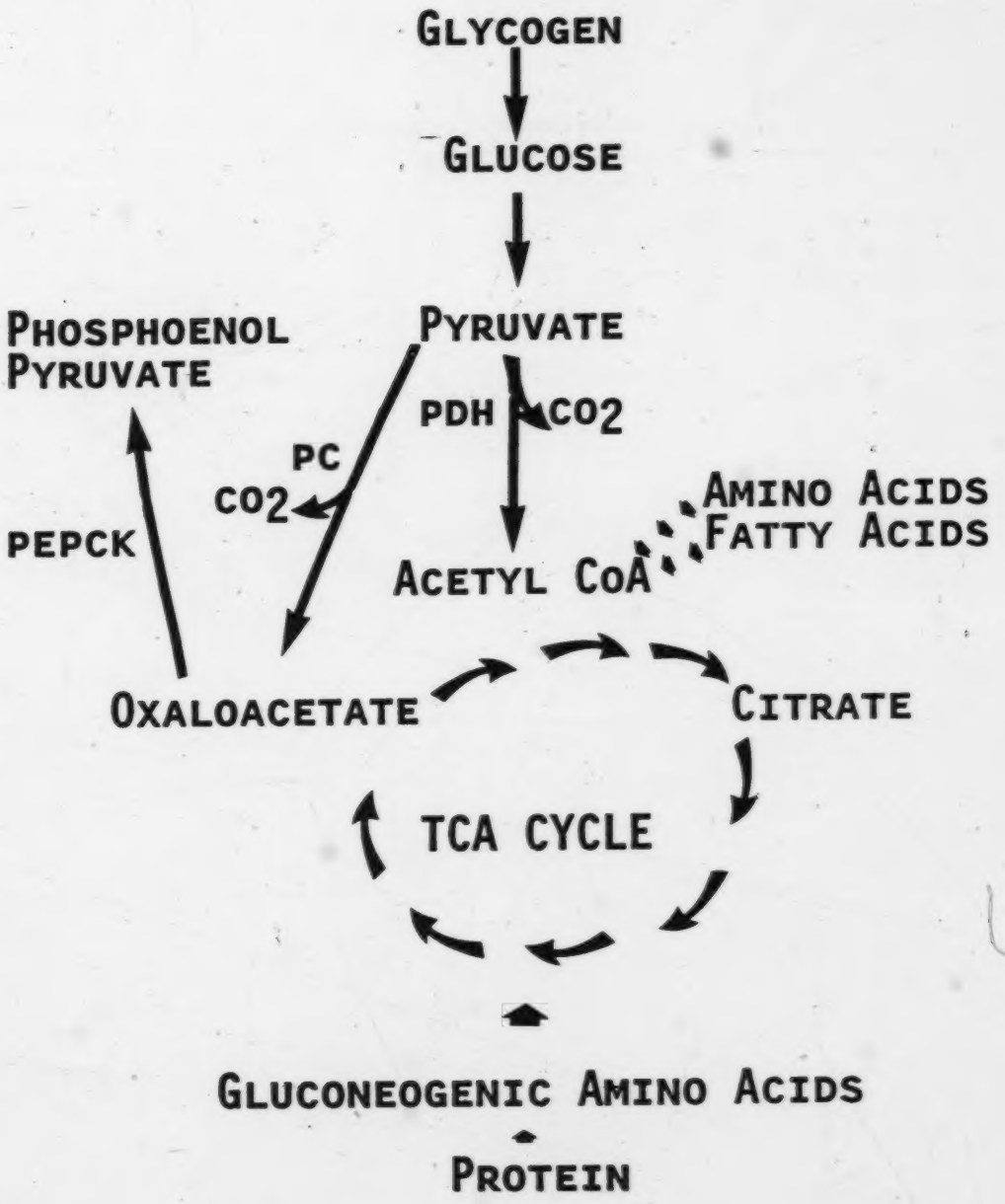


Fig. I-5: Pyruvate dehydrogenase (PDH) in the regulation of carbohydrate and fat metabolism.

PEPCK: Phosphoenolpyruvate carboxykinase.

PC: Pyruvate carboxylase.

FIGURE I.5 PYRUVATE DEHYDROGENASE (PDH) IN THE REGULATION OF FAT AND CARBOHYDRATE METABOLISM



II. MUSCLE PROTEIN TURNOVER DURING PROGRESSIVE TUMOUR GROWTH: COMPARISON OF TWO DIFFERENT EXPERIMENTAL TUMOURS IN THE RAT AND EVALUATION OF THE ROLE OF PROSTAGLANDINS.

I Materials and Methods:

a. Experimental Animals

The studies reported here were carried out in compliance with the guidelines of the Canadian Council of Animal Care. Male Sprague Dawley rats were obtained from Biosciences Animal Services, University of Alberta and were used as the host for the Yoshida Ascites Hepatoma (YAH) (Travisani et al. 1980). Inbred rats of the Buffalo strain were obtained from Sprague Dawley Co. (Harlan Sprague Dawley, Indianapolis, IN.), and were used as the host for the Morris hepatoma (MH) (Morris 1965). YAH tumours were obtained from National Cancer Institute, Fredrick, Maryland, USA; and MH were provided by the Mason Research Institute, Worcester, Massachusetts. Rats were housed in individual wire mesh cages, in a temperature (24^{o}C) and humidity (80%) controlled room. Rats were on a 12 h light:dark cycle. Rats were fed Wayne laboratory chow.

Experiments were conducted to study the effects of the two different tumors and naproxen (N) (6-Methoxy - Methyl-2 naphthaleneacetic acid), an inhibitor of prostaglandin synthesis (Brogden et al. 1979). One series of experiments was conducted with the Morris hepatoma, another series involved the

Yoshida hepatoma. Rats were allocated by initial body weight to 3 treatment groups including ad libitum fed controls and tumour bearing subjects. Since food intake is reduced in tumour - bearing rats, a pair fed control group was also included; these rats were all fed the average amount of food consumed by the tumour - bearing rats on the prior day. Pair fed rats received one meal per day at 0900 h. Rats in each treatment group received either drinking water, or water containing naproxen (76 mg/liter; approximately 11 mg/kg body weight/day). In control experiments it was determined that this represented a minimum effective dose to block fever induced by the injection of E. Coli endotoxin in the rat (see appendix).

b. Tumour Implantation

Rats were implanted either a) intraperitoneally with 0.5 ml ascites fluid containing Yoshida ascites hepatoma AH130 or b) subcutaneously in both flanks with 20 μl of finely chopped Morris hepatoma cells. Control rats were sham implanted. Rats bearing tumours were humanely sacrificed after either 8 days of implantation (YAH) or 21 days of implantation in the case of the slower growing Morris hepatoma. Both cell lines were propagated by serial transplantation in their respective hosts.

c. Muscle and Tumour Incubation

At the end of each experiment the pair fed rats received their food at 9:00 am, two hours before starting to dissect the muscles, the food from tumour and ad lib control rats were not

removed. The epitrochlearis muscle was surgically removed under general anesthesia (halothane) and incubated in vitro (Fulks et. al. 1975). The muscles were weighed and preincubated in individual flasks containing 4 ml Krebs-Ringer bicarbonate buffer medium, containing 5 mM glucose and 0.1 U/ml insulin. After 30 minutes the muscles were transfered to fresh medium for a period of 3 hours. The preincubation and incubation media were continuously gassed with O_2/CO_2 (19:1) and maintained at 37°C in a shaking water bath. The incubation media and muscle tissue were stored at -70°C for future determination of protein synthesis and net protein degradation, PGE_2 synthesis and total profile of amino acid release and intracellular amino acid content.

Two muscles, representative of slow twitch (soleus) and fast twitch (extensor digitorum longus) muscle types were also dissected and weighed. Water content was determined by drying the muscles at 100°C untill constant weight. The protein content of muscles was determined by the method of Bradford et. al. (1976). In addition, for YAH experiments the ascites fluid containing the tumour cells was removed with a syringe and the volume measured. For MH experiments the solid subcutaneous tumours were dissected and weighed.

d. Analyses

Rates of net protein degradation were determined by following the net release of tyrosine from protein into the incubation medium, and rates of protein synthesis were measured

with ^{3}H phenylalanine (0.05 μCi/ml) plus non-radioactive phenylalanine (1mM) by the method described by Tischler et al. (1981). PGE_2 production by muscles was determined by radioimmunoassay of the PGE_2 released into the incubation medium (Rodemann et al. 1982). Amino acids released into the incubation media and intracellular amino acids were separated by high performance liquid chromatography (Jones & Gilligan, 1983) using a Varian 5000 HPLC with a Fluorochrom detector. Peak area integrations were obtained by a Hewlett-Packard 2625A Computer.

All experiments were carried out with 7-10 rats / treatment and were repeated at least twice. The results for each treatment were pooled and statistically analysed by the procedures of one-way and two-way analysis of variance.

2. Results:

a. Body Weight and Food Intake

Figure II-1 shows the effects of the tumours and naproxen on food intake. The voluntary food consumption of rats bearing the Morris hepatoma was similar to that of healthy controls for 10 days, then fell gradually over the next 12 days by about 50%. In rats bearing the Yoshida hepatoma, food intake declined rapidly starting at 2 days after implantation and fell almost to zero by day 7. Naproxen had no effect on food intake in tumour - bearing or control rats.

The body weight of all rats increased over the study period (Table 1). The greatest gains were by the ad libitum fed rats (+96g MH; +49g YAH). Rats bearing tumours also gained weight, but significantly less than ad libitum fed controls. The tumour comprised a significant proportion of the gain, so that when final body weight was expressed on a tumour - free basis, YAH rats lost 14.3% of initial body weight, and MH rats gained only 5% of initial body weight. Body weight gain of rats pair fed to the tumour - bearing groups was intermediate between those of tumour - bearing and ad libitum fed rats. Treatment of healthy control rats with naproxen had no effect on body weight. In rats bearing MH, naproxen had no effect on body weight gain or tumour weight. In rats bearing YAH, naproxen treatment increased the final body weight of the tumour - bearing rats to that seen in the pair fed controls and the tumour weight was

reduced from 52 to 36 g ($p<0.05$). The final body weight of pair fed rats was also significantly increased by naproxen treatment in the YAH experiment, this was not seen in the MH experiment.

b. Muscle Weight and Protein Content

The weights of three different skeletal muscles were examined, the soleus (SOL), extensor digitorum longus (EDL) and epitrochlearis (EPI) (Table 2). Implantation with either MH or YAH, caused a significant reduction in the weights of all three muscles compared to ad libitum fed controls. The greatest decrease was seen in the EPI (-41% YAH; -30% MH), followed by the EDL (-34% YAH, -16% MH) and SOL (-27% YAH, -15% MH). The rats pair fed to YAH -bearing rats did not show any significant decrease in muscle weight, probably because of the short duration of the experiment. In rats pair fed to MH - bearing subjects, the final weight of the EPI and EDL was intermediate between the weights of muscles of tumour - bearing and ad libitum fed controls (Table II-2). By contrast, the SOL muscle of rats pair fed to MH - bearing rats did not lose weight compared to ad libitum fed controls. In general, the depression of muscle weight associated with tumour implantation was significantly greater than the effect of reduced food intake alone. In all cases the extent of muscle wasting was greater in rats implanted with YAH, compared to MH. Naproxen had no effect on the weight of muscles from ad libitum fed or pair fed rats. However, there was a striking reduction in the weight loss of all 3 muscles when rats bearing YAH were treated with naproxen,

compared to rats with YAH that did not receive naproxen ($p<0.05$). Naproxen had no effect on the muscle weights of MH - bearing rats.

In order to determine whether the muscle weights reported above were reflective of the protein levels in the different muscles, the protein content of the SOL and EDL were determined (Table II-3). For the SOL, the protein concentration (mg protein / mg muscle) was not different among treatments, so that the data on total protein shown in Table 3, reflect the trends in muscle weight seen in Table 2. By contrast, the EDL muscles showed significant variation in protein concentration. Naproxen increased the protein concentration in EDL muscles by 5-10% ($p<0.02$); tumour implantation decreased muscle protein concentration by 5% ($p<0.05$). Therefore, when data for the EDL are expressed as total protein content per muscle, the YAH was seen to significantly reduce muscle protein. Treatment with naproxen significantly increased muscle protein in tumour - bearing, pair fed and ad lib fed rats. ($p<0.01$). There was no difference in water content (EDL water content was 74% for all treatments).

c. Protein Turnover

Rates of protein synthesis and of net protein degradation in isolated epitrochlearis muscles are shown in Table II-4. Tumour implantation increased rates of net protein catabolism compared to ad libitum and pair fed controls (+64% YAH; +20% MH ($p<0.05$)). Pair feeding did not affect net protein degradation

compared to ad libitum fed controls. Treatment with naproxen virtually abolished the catabolic response to the YAH ($p<0.001$), however this agent did not substantially alter rates of net proteolysis in the ad lib, pair fed, or MH - treated rats.

Tumour implantation resulted in decreased rates of muscle protein synthesis (-38% YAH; -23% MH) (Table II-4) compared to ad libitum fed controls. Pair feeding and treatment with naproxen did not affect rates of protein synthesis in any experiment, except that rates of synthesis were significantly improved when rats implanted with YAH were treated with naproxen.

d. Prostaglandin E_2 Production

The release of PGE_2 by the incubated epitrochlearis muscle is shown in Table II-5. All muscles produced immunoreactive PGE_2 into the incubation medium (31 - 100 pg/mg muscle/3h). There was no difference in muscle PGE_2 production between ad libitum fed and pair fed rats. Muscles from tumour - bearing rats had a significantly lower PGE_2 production than the ad libitum fed rats (-68%, YAH; -22%, MH) ($P<0.05$). The administration of naproxen in the drinking water significantly reduced the production of PGE_2 by 22 - 43%, except in the case of muscles from rats bearing the YAH where there was no further reduction. The PGE_2 production of incubated YAH cells was 2.38 ± 0.36 ng / ml cells / 3 h ; when rats were treated with naproxen the PGE_2 release was inhibited to 0.44 ± 0.12 ng / ml cells / 3 h (-81%, $p<0.001$).

e. Tissue Amino Acid Levels and Amino Acid Release

i. Morris Hepatoma: Intracellular amino acids

The intracellular levels of 18 amino acids in epitrochlearis muscle are reported in Table II-6. Rats bearing the MH had lowered total intracellular levels of amino acids (-16.6%; $P<0.05$) compared to ad libitum fed controls. In the case of individual amino acids, including glu, asp, ser, his, thr, tyr, val, ile, and leu, this effect was also significant ($p<0.05$). Muscles from pair fed rats also showed decreased levels of most amino acids compared to ad libitum fed controls, notably glu, asn, his, thr, tyr, val and leu ($p<0.05$). Levels of gln were increased in pair fed rats. Although both tumour and pair fed rats showed decreased levels of intracellular amino acids, this effect was more prominent in the tumour - bearing rats, such that levels of ser, gln, his, thr, val and leu were lower in muscles from tumour - bearing rats ($p<0.05$). There was no effect of naproxen on the intracellular levels of amino acids.

ii. Morris Hepatoma: Net Amino Acid Release

The net release of 18 amino acids from isolated epitrochlearis muscles is shown in Table II-7. There were no significant differences amongst treatments in the total amino acid release or in the release rates of individual amino acids. It may be noted, however, that the net release of all amino acids was increased in tumour bearing vs pair fed controls (+16%; range +5 - +53%), although this trend did not reach

significance.

iii. Yoshida Ascites Hepatoma: Intracellular Amino Acids

The levels of intracellular amino acids in epitrochlearis muscles are shown in Table II-8. In YAH - bearing rats, levels of total amino acids fell by 35% ($P<0.05$) compared to ad libitum fed controls; levels of individual amino acids either stayed the same or fell. Levels of glu, asn, ser, gln, his, gly, thr, arg, ala, phe, and lys were significantly reduced ($p<0.05$); in the case of gln this was especially marked (-78%). This was in contrast to the pair fed rats, which showed increased levels of trp, met and phe, and decreased levels of only four amino acids (glu, gln, ala, lys); levels of other amino acids were not significantly different from ad libitum fed controls. Thus the fall in intracellular levels of amino acids was more prominent in the tumour - bearing rats compared to the pair fed controls, and levels of glu, asn, ser, gln, his, gly, thr, ala and lys were significantly less in tumour - bearing rats. Overall, naproxen did not affect the total amino acid leves on epitrochlearis muscle. In some cases, naproxen treatment increased the levels of an individual amino acid, in others a decrease was seen.

iiii. Yoshida Ascites Hepatoma: Net Amino Acid release

Implantation of the YAH significantly increased the net release of all but four amino acids from the isolated epitrochlearis muscle (Table II-9) compared to ad libitum fed controls ($p<0.05$). The total amino acid release from the tumour

- bearing rats was increased by 38.5% compared to ad libitum fed controls. By contrast, only net ala release was increased in pair fed rats compared to ad libitum fed rats; the release of glu was significantly reduced, and remaining amino acids were not significantly changed. The total amino acid release by muscles from pair fed rats was not significantly increased compared to ad libitum fed controls. Thus YAH - bearing rats showed a marked elevation of amino acid release compared to both pair fed and ad libitum fed controls. In tumour - bearing rats, the net release of virtually all individual amino acids was significantly decreased by naproxen treatment. Although a similar trend was seen in both the pair fed and the ad libitum fed rats, these differences were not always significant on an individual amino acid basis.

3. Discussion:

The results of this study show that PGE_2 production by a tumour may influence the progression of cachexia in the host. We administered naproxen, an inhibitor of Prostaglandin synthesis (Brogden et al.,1979), which was effective in decreasing PGE_2 production. For example, in control studies with rats injected with E. coli endotoxin, naproxen inhibited fever (See appendix). Results from measurements on incubated epitrochlearis muscle from rats treated with naproxen showed inhibition of PGE_2 production by approximately 40%. PGE_2 production by YAH tumour cells was also inhibited by more than 80% by naproxen treatment. Naproxen was employed in experiments with two different cachexia - inducing hepatomas, one known to produce large amounts of PGE_2 (Yoshida ascites hepatoma (Travisani et al. 1980)), and one not known to produce elevated levels of prostaglandins (Morris hepatoma). Various parameters of growth, protein deposition and protein metabolism were measured. The Morris hepatoma had no effect on muscle PGE_2 production, and administration of naproxen had no effect on tumour size, muscle size or protein content, protein degradation or protein synthesis. Thus it would appear that the MH induces cachexia in the host in a prostaglandin - independant fashion.

The YAH cells produced large amounts of PGE_2 (2.38ng PGE_2 / ml ascites fluid / 3h), compared to the normal PGE_2 production by host muscle tissue in the picogram range. If the values for PGE_2 production by the tumour in vitro are multiplied by the tumour size, it is possible to estimate that

the tumour would result in an additional 41 ng PGE_2 produced per h on a whole body basis. This may account for the apparent down - regulation of PGE_2 production by muscles of rats bearing the YAH (68% below control levels before naproxen treatment). Inhibition of prostaglandin synthesis in rats bearing YAH had dramatic effects on several aspects of growth and protein metabolism. Although naproxen had no effect on the feed intake of YAH - bearing rats, the body weight loss induced by the tumour was inhibited by 64% by this agent. In parallel with body weight, soleus, EDL and epitrochlearis muscles from YAH bearing rats showed significant atrophy and protein loss; this effect was inhibited by about 40% by administration of naproxen. Therefore, our data suggests that 40% of the muscle wasting observed was due to the high levels of PGE_2 produced by the YAH cells. The residual 60 % of muscle wasting could be partly due to PGE_2 released by YAH cells (which was not totally inhibited by naproxen), and/or other factors (see below). The loss of muscle tissue protein appeared to be due to increased protein degradation and decreased protein synthesis. Administration of naproxen resulted in a substantial reduction in the net loss of all amino acids from the incubated epitrochlearis muscle. In addition, naproxen returned rates of protein synthesis and net degradation to levels seen in healthy controls. Finally, our studies and those of other authors (Travisani et al. 1980) have shown that the use of a PGE_2 synthesis inhibitor in YAH bearing rats decreases the tumour mass by 33% to 36%. The results obtained in this study concur

with others, suggesting a role for prostaglandins in regulation of protein metabolism in physiological and pathological states (Fagan et al.,1985; Ruff & Secrist,1984). It is also of some interest that naproxen treatment for 7 days increased the protein content of EDL but not soleus muscles from healthy ad libitum or pair fed controls. Although this aspect requires further investigation, it is possible that a component of normal muscle growth in the rat may be prostaglandin - dependant.

Prostaglandin production by tumours has several clinically important side effects including bone resorption, immunosupression and promotion of tumour initiation and growth (Fig II-2) (Garaci et al. 1987). PGE_2 is a catabolic factor for bone, causing breakdown of this tissue and secondary hypercalcemia (Voelkel et al. 1975; Tashjian et al. 1974); this condition can be improved by administration of a prostaglandin synthesis inhibitor such as aspirin or indomethacin, and the elevated levels of Ca^{2+} in plasma resulting from bone resorption can be decreased to control levels (Tashjian et al. 1974; Seyberth et al. 1977; Voelkel et al. 1975). Prostaglandins of the E series act physiologically as feedback inhibitors of cellular and humoral immune responses including T cell proliferation, lymphokine production and cytotoxicity, and macrophage and natural killer cytotoxicity (Goodwin & Ceuppens 1983). Similarly, in tumours which produce high levels of prostaglandins and secondary immunosupression, indomethacin has been shown to greatly decrease the immunosupressive effect (Goodwin & Ceuppens 1983). Finally, prostaglandins have been

linked to carcinogenesis and to the proliferation and differentiation of some types of tumour cells, and aspirin-like drugs inhibit these processes (Garaci et al. 1987). Based on the present results, muscle protein catabolism may be added to the catalogue of deleterious side effects of tumours that produce high levels of PGE_2. The use of inhibitors of prostaglandin synthesis could be effective to help maintain lean body mass in patients with tumours that produce PGE_2, while they receive other treatments such as chemotherapy and radiotherapy.

The cause of muscle wasting in tumour bearing rats is complex and has not been fully clarified. As in a number of related studies (Lundholm et al.,1981; Kawamura et al. 1982; Tayek et al. 1986), pair fed controls were included in order to clarify the component of wasting attributable to the loss of feed intake. In the rats pair fed to subjects implanted with the YAH, no significant loss of muscle weight or protein content was observed. The absence of any detectable effect may have been due to the rather short term food deprivation. By contrast, about 40% of the loss of weight of fast twitch muscles seen in rats bearing the MH was attributable to the loss of feed intake, as judged by pair fed controls. The slow twitch soleus muscle did not weigh less in pair fed versus ad libitum fed controls. Thus the extent of muscle weight loss due to decreased feed intake depends upon the fibre type of the muscles affectedas well as the type of tumour and possibly the duration of its development. As shown above, in rats bearing the YAH, about 40% of the muscle loss in soleus, EDL and epitrochlearis

appeared to be prostaglandin - dependant. PGE_2 released by the YAH may cause muscle wasting directly or indirectly (Fig. 2-3). There is evidence for a direct effect of PGE_2 on isolated muscle, causing an increase in protein degradation (Rodemann & Goldberg, 1982; Reeds et al., 1983). However, elevated levels of PGE_2 in tumour - bearing individuals may also cause bone resorption and hypercalcemia. High levels of Ca^{2+} activate protein degradation in skeletal muscle (Baracos et al.,1984b) and Ca^{2+} may indirectly result in the prominent muscle wasting seen in the YAH - bearing rats. Finally, the action of other catabolic factors produced by the host or the tumour could be enhanced by PGE_2, causing an increase in muscle catabolism. Since in both tumour models employed in this study about 60% of the muscle wasting observed could not be explained, these other factors are likely to be very important in the development of cachexia.

Both experimental tumours employed in this investigation caused modifications in the growth and metabolism of skeletal muscle. The growth of three different muscles representing two different fibre types was inhibited in tumour - bearing rats. The magnitude of the response varied with tumour and muscle type. In the YAH, soleus, EDL and epitrochlearis muscles were about 40% lighter than in ad lib fed controls. In the MH, the epitrochlearis was most severely affected (-29%), followed by the EDL (-20%) and the soleus (-15%). The slow twitch soleus was less affected in MH - bearing rats and actually increased significantly in size as a proportion of body weight. There is

some evidence that changes in protein synthesis and breakdown under different conditions differs with muscle fiber type (Waterlow 1984).

Rates of protein synthesis and degradation in the epitrochlearis muscle were affected by tumour implantation. Rates of protein synthesis were inhibited in rats bearing both tumours (-38% in YAH and -23% in MH). Protein degradation was increased in epitrochlearis muscles from rats bearing YAH by 38%; MH implantation did not affect rates of muscle protein degradation. The net result of these changes was increased amino acid release from muscle tissues. The loss of amino acids from the tissue was proportional to the severity of the wasting seen, so that the effects of the YAH on protein synthesis, degradation and net amino acid release were consistently greater than those of the MH. Although muscles incubated in vitro are always in a net catabolic state, even if they originate from growing rats (Baracos & Goldberg, 1986.), it is encouraging that results obtained in vitro concur qualitatively and quantitatively with the effects observed in vivo. An interesting point is that although pair feeding in the MH experiments caused significant atrophy of the epitrochlearis muscle over the three weeks following implantation, the changes in muscle protein synthesis and degradation that account for this loss were too small to measure in this preparation. A variety of other authors have examined protein turnover in muscles of experimental animals bearing different tumours (Tessitore et al. 1987; Goodlad et al. 1981; Lundholm et al. 1976-81-82;

Benegard et al. 82; Burt et al. 1984; Norton et al. 1984; Kawamura et al. 1982; Emery et al. 1984a-b; Pain et al. 1984; Tayek et al. 1986). Although many different models and methods of determination have been used, there is general agreement that protein synthesis falls and/or protein degradation rises in muscles of tumour - bearing individuals. Our results suggest that the contribution of protein synthesis and degradation to wasting may vary with tumour type, since the YAH altered rates of both processes and the MH only appeared to affect protein synthesis. It is not clear why the results of Tessitore et al. (1987) on rats bearing YAH differ from those we obtained. Using [^{14}C]-bicarbonate as precursor, they showed that only rates of protein degradation in YAH - bearing rats were increased and protein synthesis was unchanged.

Data obtained from analysis of intracellular and exported amino acids from incubated epitrochlearis muscles in the present study suggest several alterations of protein and amino acid metabolism in muscles of feed - restricted and tumour - bearing rats. Feed restriction resulted in relatively minor changes in levels of intracellular amino acids, and in the net release of amino acids from tissues. Changes in net amino acid release and intracellular amino acids were not identical for the two pair feeding protocols, suggesting differences in muscle amino acid metabolism between acute and long term food deprivation. For example, net alanine release was increased in rats pair fed to YAH group, but not in rats pair fed to MH. By contrast to pair fed controls, the effects of tumour implantation on both

intracellular levels and net export of amino acids was very prominent, and especially so in the case of the Yoshida hepatoma. This suggests unique aspects of amino acid utilization in the tumour - bearing host which may arise both from the tumour and from the host effort to compensate for its presence. It is perhaps noteworthy that the patterns of net amino acid release seen in tumour - bearing rats and pair fed controls in the present study are both quantitativly and qualitatively similar to those reported for the net flux of amino acids across the leg of malnourished cancer patients and malnourished controls (Bennegard et al. 1984). Thus isolated muscle may represent a useful experimental system for study of altered amino acid export in the tumour - bearing state.

Free amino acids within muscle have two major potential fates: precursors for the synthesis of tissue protein, or in the case of leucine, isoleucine, valine, aspartate, glutamate and alanine, they can be oxidized in muscle via entry to the TCA cycle (Goodlad et al. 1981; Tawa, & Golberg, 1986). A third fate of amino acids in skeletal muscle is exportation to supply metabolic processes elsewhere in the body. For example, alanine is used extensively for hepatic gluconeogenesis, whereas glutamine may be converted to glucose in kidney. The amino acids released by skeletal muscle may be used by the tumour for oxidation and protein synthesis; amino acids are also donors of carbon (serine) and amino N groups (glutamine) for synthesis of pyrimidines, purines and nucleotides which are essential for tumour cell proliferation (Medina et al. 1988). Recently it has

become apparent that some tumours may utilize specific amino acids as preferred sources of metabolic energy. For example, Rivera et al. (1988) using Lewis lung carcinoma, showed that glutamine is exported by skeletal muscle to the liver and tumour, and that glutamine is used by the tumour for oxidation for energy and incorporation into the tumour tissue. Similarly, the Morris hepatoma 7777 has been shown to prefer glutamine for oxidative metabolism (Mares-Perlman & Shrago, 1988). Our data was consistent with the above studies, and showed a much larger decrease in intracellular glutamine (-52%) than in any other amino acid and an increase in glutamine release (+48%) from epitrochlearis muscle of YAH - bearing rats compared to pair fed controls. Although not examined in the present study, it seems likely that YAH may be a glutamine utilizing tumour. The MH - bearing rats also showed a decrease in intracellullar glutamine (-43%); but no detectable change in glutamine release from epitrochlearis muscle compared to pair fed controls. Since Rennie and coworkers (MacLennan et al. 1987) have suggested that gln may directly activate protein synthesis in muscle, the severe fall in intracellular gln seen here may also have importance for the pathogenesis of muscle atrophy. It would appear that studies of the amino acid utilization by tumours would be essential to fully assess the ramifications of cancer on protein metabolism in the whole body.

Table II-1. Effects of Tumours and Naproxen (N) on Whole Body and Tumour Growth

Treatment	Initial Body Wt	Final Body Wt	Tumour Wt Weight (g)	Tumour Free Body Wt Gain	Tumour-free Body
Yoshida Ascites Hepatoma AH130					
Ad Libitum fed	266	315^{a}		315^{a}	$+49^{a}$
Tumour	266	279^{bc}	52.3^{a}	228^{b}	-38^{b}
Pair Fed	266	270^{b}		270^{c}	$+4^{c}$
Ad Lib + N	266	318^{a}		319^{a}	$+52^{a}$
Tumour + N	266	320^{a}	35.6^{b}	284^{cd}	$+18^{d}$
Pair Fed + N	266	295^{ac}		295^{d}	$+29^{d}$
Morris Hepatoma 7777					
Ad libitum fed	198	294^{a}		294^{a}	$+96^{a}$
Tumour	198	237^{b}	29	205^{b}	$+10^{b}$
Pair Fed	198	248^{b}		250^{c}	$+50^{c}$
Ad lib + N	198	287^{a}		287^{a}	$+89^{a}$
Tumour + N	198	241^{b}	35	206^{b}	$+8^{b}$
Pair Fed + N	198	250^{b}		248^{c}	$+52^{c}$

Numbers with different superscripts in the same column are significantly different ($p<0.05$; $n=9$)

Male rats were implanted with either the Yoshida ascites hepatoma AH130 or the Morris hepatoma 7777. Rats were provided water or water containing naproxen (N) (71 mg/l) ad libitum. Pair fed rats were offered the same amount of food as the corresponding tumour - bearing rats had eaten on the prior day. Rats were sacrificed 7 days (YAH) and 21 days (MH) after implantation.

Table II.2. Effect of Tumours and Naproxen on Muscle Weights

Treatment	Muscle Weight (mg) Epitrochlearis	Soleus	EDL
Yoshida Ascites Hepatoma AH130			
Ad Libitum fed	69.4^{a}	131.31^{a}	148.1^{a}
Tumour	40.9^{b}	95.4^{b}	98.0^{b}
Pair Fed	70.3^{a}	131.9^{a}	138.5^{a}
Ad lib + N	73.4^{a}	139.5^{a}	137.3^{a}
Tumour + N	53.0^{c}	111.1^{c}	116.3^{c}
Pair Fed + N	66.8^{a}	132.5^{a}	146.0^{a}
Morris Hepatoma 7777			
Ad Libitum fed	79.8^{a}	107.9^{ac}	135.2^{a}
Tumour	56.1^{b}	91.4^{b}	108.8^{bc}
Pair Fed	69.4^{cd}	114.4^{a}	123.6^{c}
Ad lib + N	73.5^{cd}	102.4^{bc}	131.0^{a}
Tumour + N	54.5^{b}	91.8^{b}	104.0^{b}
Pair Fed + N	63.1^{c}	96.8^{b}	119.8^{c}

Numbers with different superscripts in the same column are significantly different ($p<0.05$; $n=9$)

Male rats were implanted with either the Yoshida ascites hepatoma AH130 or the Morris hepatoma 7777. Rats were provided water or water containing naproxen (N) (71 mg/l) ad libitum. Pair fed rats were offered the same amount of feed as the corresponding tumour - bearing rats had eaten on the prior day. Rats were sacrificed 7 days (YAH) and 21 days (MH) after implantation.

Table II.3. Effect of Yoshida Ascites Hepatoma And Naproxen on Muscle Protein content

Treatment	Total Muscle Protein (mg) Soleus	EDL
Yoshida Ascites Hepatoma AH130		
Ad Libitum fed	7.26^{c}	13.92^{c}
Tumour	5.09^{a}	7.93^{a}
Pair Fed	6.77^{c}	13.27^{c}
Ad lib + N	7.42^{c}	15.59^{d}
Tumour + N	6.12^{b}	10.43^{b}
Pair Fed + N	7.08^{c}	14.91^{d}

Numbers with different superscripts in the same column are significantly different ($p<0.05$; n=9 per treatment)

Male rats were implanted with the Yoshida ascites hepatoma AH130. Rats were provided water or water containing naproxen (N) (71 mg/l) ad libitum. Pair fed rats were offered the same amount of food as the corresponding tumour - bearing rats had eaten on the prior day. Rats were sacrificed after 7 days and muscles dissected for the determination of protein content. Total protein soluble in 1M NaOH was determined by the method of Bradford (1976).

Table II.4. Net Protein Degradation and Protein Synthesis in Isolated Epitrochlearis Muscle

Treatment	Net Protein Degradation	Protein Synthesis	Protein Degradation*
	(nmol phe/mg muscle/3h		
Yoshida Ascites Hepatoma AH130			
Ad Libitum fed	0.595[a]	0.154[a]	0.672
Tumour	0.975[b]	0.095[b]	1.083
Pair Fed	0.602[a]	0.147[a]	0.710
Ad lib + Naproxen	0.508[a]	0.195[a]	0.650
Tumour + Naproxen	0.648[a]	0.143[a]	0.707
Pair Fed + Naproxen	0.473[a]	0.194[a]	0.568
Morris Hepatoma 7777			
Ad Libitum fed	0.255[a]	0.219[b]	0.474
Tumour	0.305[b]	0.168[ab]	0.473
Pair Fed	0.233[a]	0.213[ab]	0.446
Ad lib + Naproxen	0.253[a]	0.201[ab]	0.453
Tumour + Naproxen	0.253[a]	0.183[a]	0.436
Pair Fed + Naproxen	0.249[a]	0.183[ab]	0.432

Numbers with different superscripts in the same column are significantly different ($p<0.05$; $n\geq14$).

Male rats were implanted with either the Yoshida ascites hepatoma AH130 or the Morris hepatoma 7777. Rats were provided water or water containing naproxen (N) (71 mg/l) ad libitum. Pair fed rats were offered the same amount of feed as the corresponding tumour - bearing rats had eaten on the prior day. Rats were sacrificed after 7 days (YAH) and 21 days (MH) of implantation. * Calculated value = protein synthesis + net degradation.

Table II.5. Effect of Tumours and Naproxen on PGE_2 Production by Incubated Epitrochlearis Muscles

Treatment	PGE_2 Production (pg / mg muscle / 3 h)
Yoshida Ascites Hepatoma AH130	
Ad Libitum fed	98.8^a
Tumour	31.9^b
Pair Fed	86.7^a
Ad lib + N	58.1^b
Tumour + N	35.8^b
Pair Fed + N	54.7^b
Morris Hepatoma 7777	
Ad Libitum fed	79.6^b
Tumour	61.9^c
Pair Fed	73.4^b
Ad lib + N	62.3^c
Tumour + N	41.0^a
Fair Fed + N	53.6^c

Numbers with different superscripts in the same column are significantly different ($p<0.05$; n=8 or 9 per treatment)

Male rats were implanted with either the Yoshida ascites hepatoma AH130 or the Morris hepatoma 7777. Rats were provided water or water containing naproxen (N) (71 mg/l) ad libitum. Pair fed rats were offered the same amount of food as the corresponding tumour - bearing rats had eaten on the prior day. Rats were sacrificed after 7 days (YAH) and 21 days (MH) of implantation, and the epitrochlearis muscle removed for incubation.

Table II.6. Effects of Morris Hepatoma and Naproxen on Intracellular Amino Acid Levels in Epitrochlearis Muscle.

(nmoles of amino acid/mg of muscle)

	Naproxen	ad lib Fed	Tumour	Pair Fed
Asp	-	0.174^{ab}	0.166^{ab}	0.145^{ab}
	+	0.170^{ab}	0.179^{a}	0.132^{b}
Glu	-	0.650^{a}	0.441^{cd}	0.477^{c}
	+	0.628^{a}	0.398^{d}	0.449^{cd}
Asn	-	0.175^{b}	0.122^{a}	0.136^{a}
	+	0.171^{b}	0.123^{a}	0.134^{a}
Ser	-	0.606^{b}	0.423^{a}	0.554^{b}
	+	0.567^{b}	0.422^{a}	0.549^{b}
Gln	-	1.169^{a}	0.944^{a}	1.660^{b}
	+	1.487^{b}	0.825^{a}	1.843^{b}
His	-	0.193^{a}	0.132^{b}	0.164^{c}
	+	0.204^{a}	0.124^{b}	0.159^{c}
Gly	-	2.561^{a}	2.158^{a}	2.503^{a}
	+	2.355^{a}	2.356^{a}	2.101^{a}
Thr	-	0.385^{c}	0.259^{a}	0.312^{b}
	+	0.372^{c}	0.238^{a}	0.304^{b}
Arg	-	1.247^{ab}	1.129^{ab}	1.110^{ab}
	+	1.321^{a}	1.235^{ab}	1.043^{b}
Ala	-	1.042^{a}	0.839^{a}	1.055^{a}
	+	1.108^{a}	1.102^{a}	0.903^{a}
Tyr	-	0.131^{b}	0.107^{a}	0.104^{a}
	+	0.138^{b}	0.094^{a}	0.106^{a}
Trp	-	0.041^{a}	0.040^{a}	0.035^{a}
	+	0.043^{a}	0.039^{a}	0.038^{a}
Met	-	0.036^{a}	0.010^{b}	0.023^{ab}
	+	0.036^{a}	0.019^{ab}	0.022^{ab}
Val	-	0.140^{c}	0.084^{a}	0.105^{b}
	+	0.142^{c}	0.064^{a}	0.117^{b}
Phe	-	0.100^{b}	0.087^{ab}	0.087^{ab}
	+	0.105^{b}	0.072^{a}	0.092^{ab}
Ile	-	0.103^{a}	0.073^{bc}	0.081^{b}
	+	0.106^{a}	0.060^{c}	0.089^{ab}
Leu	-	0.172^{ab}	0.122^{c}	0.140^{b}
	+	0.176^{a}	0.102^{c}	0.152^{ab}
Lys	-	0.360^{a}	0.296^{a}	0.270^{a}
	+	0.369^{a}	0.309^{a}	0.281^{a}
TOTAL	-	9.276^{b}	7.434^{a}	8.962^{ab}
	+	9.501^{b}	7.762^{a}	8.516^{b}

Numbers with different superscripts within the same amino acid are significantly different ($P<0.05$; n=8)

Table II.7. Effects of Morris Hepatoma and Naproxen on Amino Acid Release From Isolated Epitrochlearis Muscle.
(nmol amino acid released/mg muscle/3h)

	Naproxen	Ad Lib Fed	Tumour	Pair Fed
Asp	-	0.078^{a}	0.082^{a}	0.068^{a}
	+	0.085^{a}	0.062^{a}	0.104^{a}
Glu	-	0.323^{ac}	0.277^{ab}	0.247^{bc}
	+	0.338^{ac}	0.217^{b}	0.354^{a}
Asn	-	0.196^{ab}	0.204^{ab}	0.154^{a}
	+	0.202^{ab}	0.193^{ab}	0.217^{b}
Ser	-	0.552^{a}	0.672^{ab}	0.586^{ab}
	+	0.594^{ab}	0.635^{ab}	0.741^{b}
Gln	-	2.208^{ac}	1.922^{bc}	1.835^{b}
	+	2.419^{a}	1.661^{b}	2.415^{a}
His	-	0.278^{a}	0.254^{ab}	0.207^{b}
	+	0.281^{a}	0.227^{ab}	0.244^{ab}
Gly	-	1.235^{a}	1.332^{a}	1.171^{a}
	+	1.233^{a}	1.253^{a}	1.471^{a}
Thr	-	0.540^{a}	0.582^{a}	0.525^{a}
	+	0.546^{a}	0.534^{a}	0.593^{a}
Arg	-	0.487^{a}	0.404^{a}	0.357^{a}
	+	0.421^{a}	0.390^{a}	0.374^{a}
Ala	-	1.035^{a}	1.210^{ab}	1.055^{a}
	+	1.061^{a}	1.429^{b}	1.252^{ab}
Tyr	-	0.238^{a}	0.257^{a}	0.187^{b}
	+	0.245^{a}	0.248^{a}	0.245^{a}
Trp	-	0.080^{ab}	0.087^{b}	0.070^{a}
	+	0.084^{ab}	0.089^{b}	0.094^{ab}
Met	-	0.131^{ab}	0.165^{b}	0.108^{a}
	+	0.141^{ab}	0.168^{b}	0.141^{ab}
Val	-	0.368^{a}	0.356^{a}	0.275^{a}
	+	0.372^{a}	0.314^{a}	0.388^{a}
Phe	-	0.235^{a}	0.257^{a}	0.189^{b}
	+	0.245^{a}	0.244^{a}	0.261^{a}
Ile	-	0.227^{a}	0.256^{a}	0.183^{b}
	+	0.248^{a}	0.246^{a}	0.237^{a}
Leu	-	0.442^{a}	0.459^{ab}	0.360^{b}
	+	0.471^{a}	0.452^{ab}	0.445^{ab}
Lys	-	0.708^{a}	0.716^{a}	0.576^{a}
	+	0.705^{a}	0.733^{a}	0.598^{a}
TOTAL	-	9.703^{a}	9.491^{a}	8.154^{a}
	+	9.360^{a}	9.906^{a}	10.17^{a}

Numbers with different superscripts within the same amino acid are significantly different ($P<0.05$; n=8)

Table II.8. Effects of Yoshida Ascites Hepatoma and Naproxen On Intracellular Amino Acid Concentration in Epitrochlearis Muscle. (nmoles of amino acid/mg of muscle)

	Naproxen	Ad Lib Fed	Tumour	Pair Fed
Asp	-	0.131^{bc}	0.146^{ac}	0.157^{a}
	+	0.124^{bc}	0.113^{b}	0.126^{b}
Glu	-	0.478^{c}	0.338^{a}	0.361^{a}
	+	0.465^{c}	0.288^{b}	0.391^{a}
Asn	-	0.145^{b}	0.103^{a}	0.152^{b}
	+	0.149^{b}	0.113^{a}	0.149^{b}
Ser	-	0.447^{b}	0.273^{a}	0.481^{b}
	+	0.449^{b}	0.278^{a}	0.441^{b}
Gln	-	1.766^{a}	0.385^{c}	0.797^{c}
	+	1.639^{a}	0.523^{c}	1.228^{d}
His	-	0.181^{c}	0.076^{a}	0.164^{c}
	+	0.166^{c}	0.110^{b}	0.169^{c}
Gly	-	1.751^{b}	0.653^{a}	1.776^{b}
	+	1.729^{b}	0.848^{a}	1.966^{b}
Thr	-	0.277^{d}	0.117^{a}	0.287^{d}
	+	0.304^{d}	0.184^{b}	0.238^{abc}
Arg	-	1.388^{b}	2.191^{a}	1.617^{b}
	+	1.571^{b}	2.027^{ac}	1.741^{bc}
Ala	-	0.795^{b}	0.421^{a}	1.351^{c}
	+	0.878^{b}	0.576^{a}	1.106^{d}
Tyr	-	0.145^{a}	0.114^{a}	0.144^{a}
	+	0.143^{a}	0.125^{a}	0.135^{a}
Trp	-	0.035^{a}	0.046^{ab}	0.060^{b}
	+	0.037^{ab}	0.038^{ab}	0.049^{ab}
Met	-	0.043^{a}	0.041^{a}	0.066^{b}
	+	0.041^{a}	0.037^{a}	0.038^{a}
Val	-	0.128^{a}	0.110^{a}	0.148^{a}
	+	0.136^{a}	0.117^{a}	0.119^{a}
Phe	-	0.129^{c}	0.223^{a}	0.178^{b}
	+	0.167^{bc}	0.167^{b}	0.177^{b}
Ile	-	0.117^{a}	0.105^{a}	0.135^{a}
	+	0.125^{a}	0.104^{a}	0.114^{a}
Leu	-	0.157^{a}	0.120^{a}	0.164^{a}
	+	0.163^{a}	0.127^{a}	0.150^{a}
Lys	-	0.518^{c}	0.107^{a}	0.312^{b}
	+	0.428^{d}	0.252^{b}	0.425^{dc}
TOTAL	-	8.631^{a}	5.571^{b}	8.349^{a}
	+	8.714^{a}	6.028^{b}	8.757^{a}

Numbers with different superscripts within the same amino acid are significantly different ($P<0.05$; n=8)

Table II.9. Effects of Yoshida Hepatoma and Naproxen on Amino Acid Release From Isolated Epitrochlearis Muscle.

		(nmol amino acid released/mg muscle/3h)		
	Naproxen	Ad Lib Fed	Tumour	Pair Fed
Asp	-	0.189^{a}	0.143^{a}	0.181^{a}
	+	0.179^{a}	0.122^{a}	0.139^{a}
Glu	-	0.709^{bc}	0.772^{a}	0.566^{c}
	+	0.624^{c}	0.452^{a}	0.443^{d}
Asn	-	0.358^{c}	0.698^{a}	0.375^{c}
	+	0.338^{c}	0.545^{b}	0.342^{c}
Ser	-	1.215^{ab}	1.489^{a}	1.332^{ab}
	+	1.084^{b}	1.126^{b}	1.025^{b}
Gln	-	2.846^{c}	3.608^{a}	1.951^{b}
	+	2.573^{c}	3.163^{ac}	2.046^{b}
His	-	0.540^{c}	0.887^{a}	0.558^{c}
	+	0.466^{bc}	0.523^{c}	0.368^{b}
Gly	-	2.834^{a}	2.694^{ab}	2.469^{ab}
	+	2.226^{ab}	2.129^{b}	2.313^{ab}
Thr	-	1.121^{b}	1.567^{a}	1.041^{b}
	+	0.874^{c}	1.118^{b}	0.832^{c}
Arg	-	0.658^{b}	1.078^{a}	0.558^{b}
	+	0.760^{b}	0.609^{b}	0.600^{b}
Ala	-	2.412^{ab}	3.146^{a}	3.899^{c}
	+	2.178^{b}	2.421^{b}	2.554^{ab}
Tyr	-	0.595^{a}	0.975^{b}	0.602^{a}
	+	0.508^{a}	0.648^{a}	0.473^{a}
Trp	-	0.153^{c}	0.278^{a}	0.185^{c}
	+	0.142^{c}	0.231^{b}	0.156^{c}
Met	-	0.267^{c}	0.639^{a}	0.314^{bc}
	+	0.235^{c}	0.382^{b}	0.223^{c}
Val	-	0.673^{a}	1.170^{b}	0.691^{a}
	+	0.609^{a}	0.723^{a}	0.501^{a}
Phe	-	0.518^{bc}	0.988^{a}	0.563^{b}
	+	0.455^{bc}	0.564^{b}	0.374^{c}
Ile	-	0.495^{a}	0.841^{b}	0.496^{a}
	+	0.444^{a}	0.507^{a}	0.372^{a}
Leu	-	0.849^{a}	1.352^{b}	0.842^{a}
	+	0.761^{a}	0.822^{a}	0.600^{a}
Lys	-	1.416^{a}	2.409^{b}	1.595^{a}
	+	1.267^{a}	1.285^{a}	1.170^{a}
TOTAL	-	17.85^{b}	24.74^{a}	18.21^{b}
	+	15.72^{b}	17.37^{b}	14.53^{b}

Numbers with different superscripts within the same amino acid are significantly different ($P<0.05$; $n=8$)

FIG.II.1A

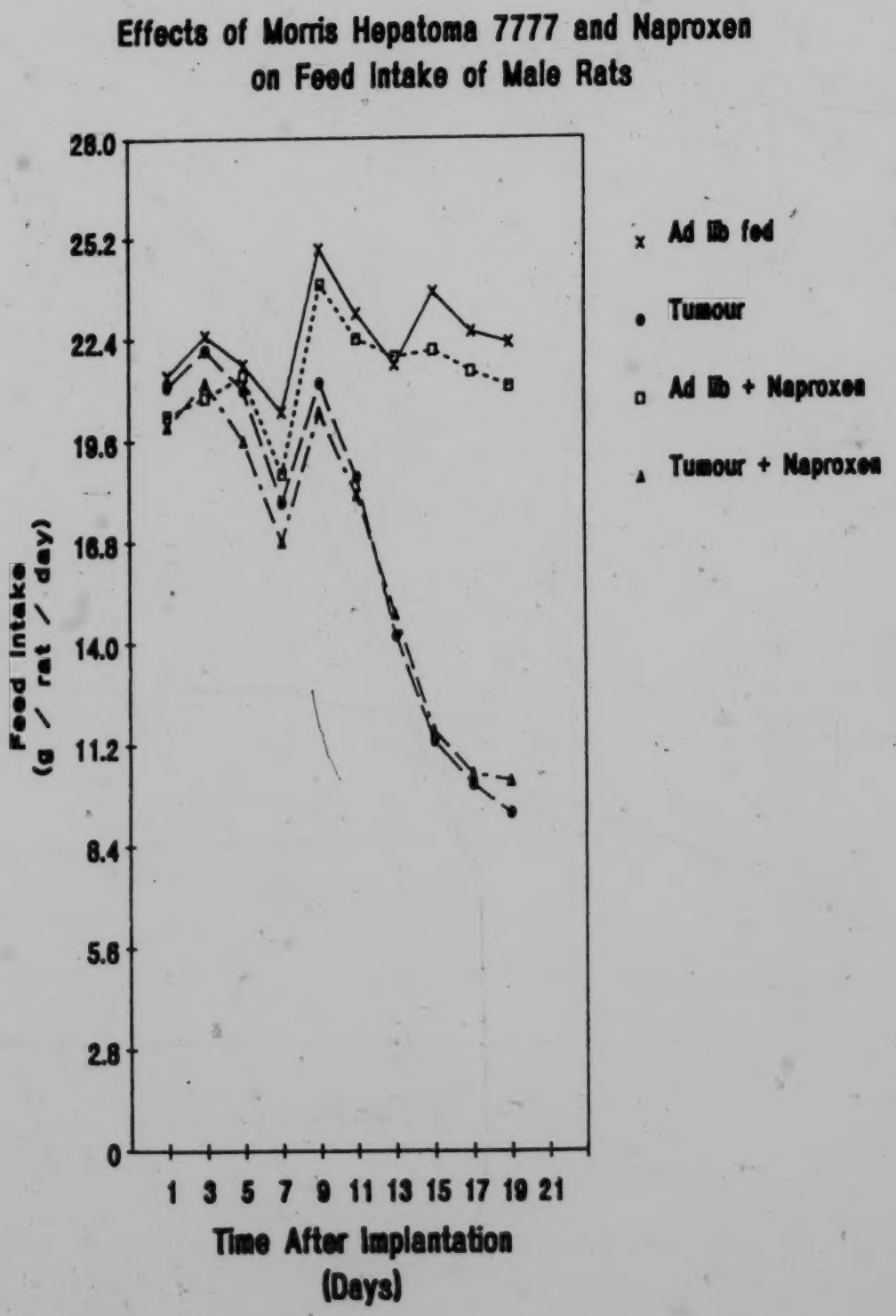

Effects of Morris Hepatoma 7777 and Naproxen
on Feed Intake of Male Rats
28.0
25.2
22.4
19.6
16.8
14.0
11.2
8.4
5.6
2.8
0
Feed Intake
(g / rat / day)
1 3 5 7 9 11 13 15 17 19 21
Time After Implantation
(Days)
Ad lib fed
Tumour
Ad lib + Naproxen
Tumour + Naproxen

FIG.II.1B

Effects of Yoshida Ascites Hepatoma and Naproxen on Feed Intake of Male Rats

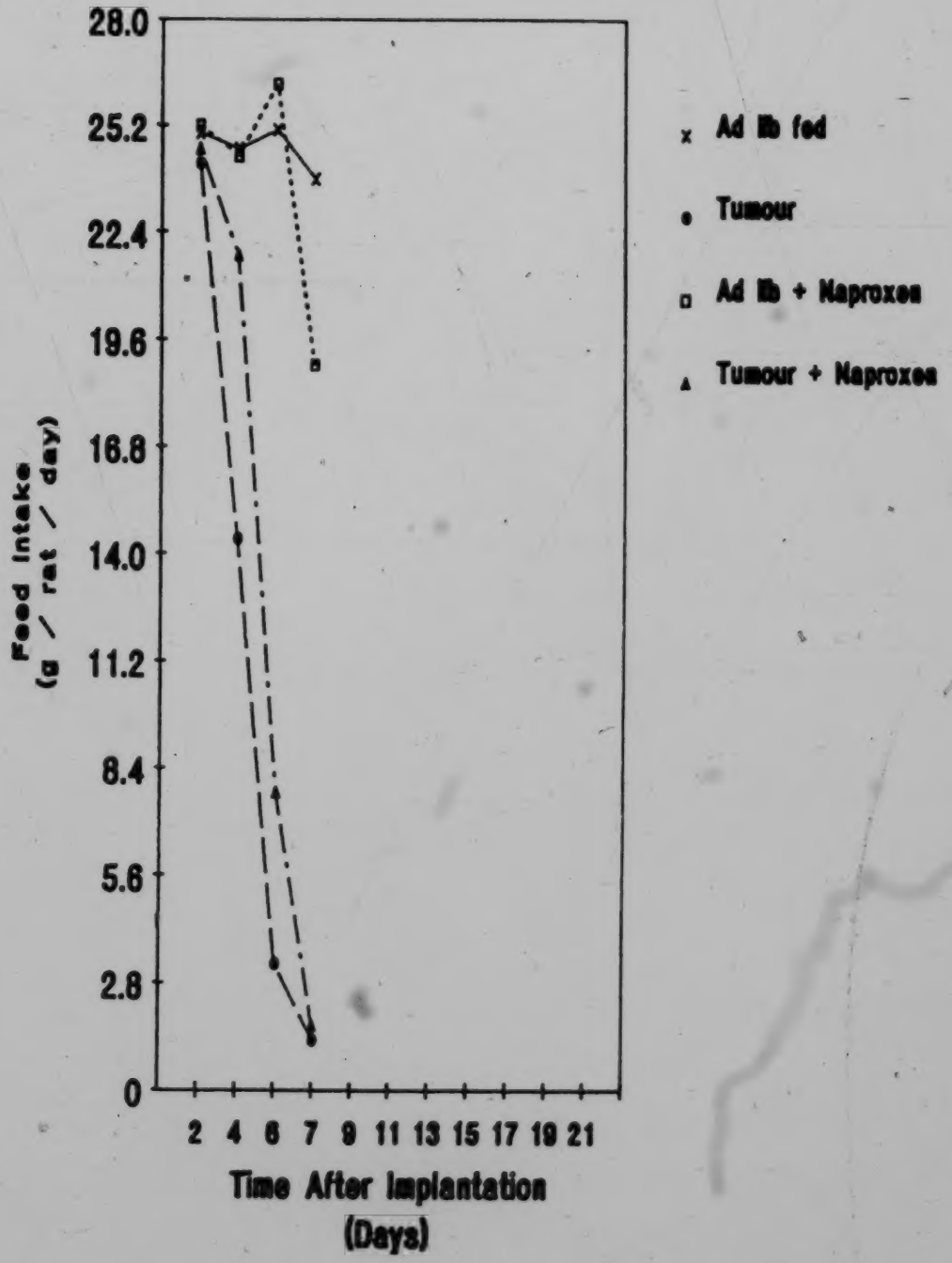


Figure II.2 EFFECTS OF TUMOUR PGE_2 PRODUCTION

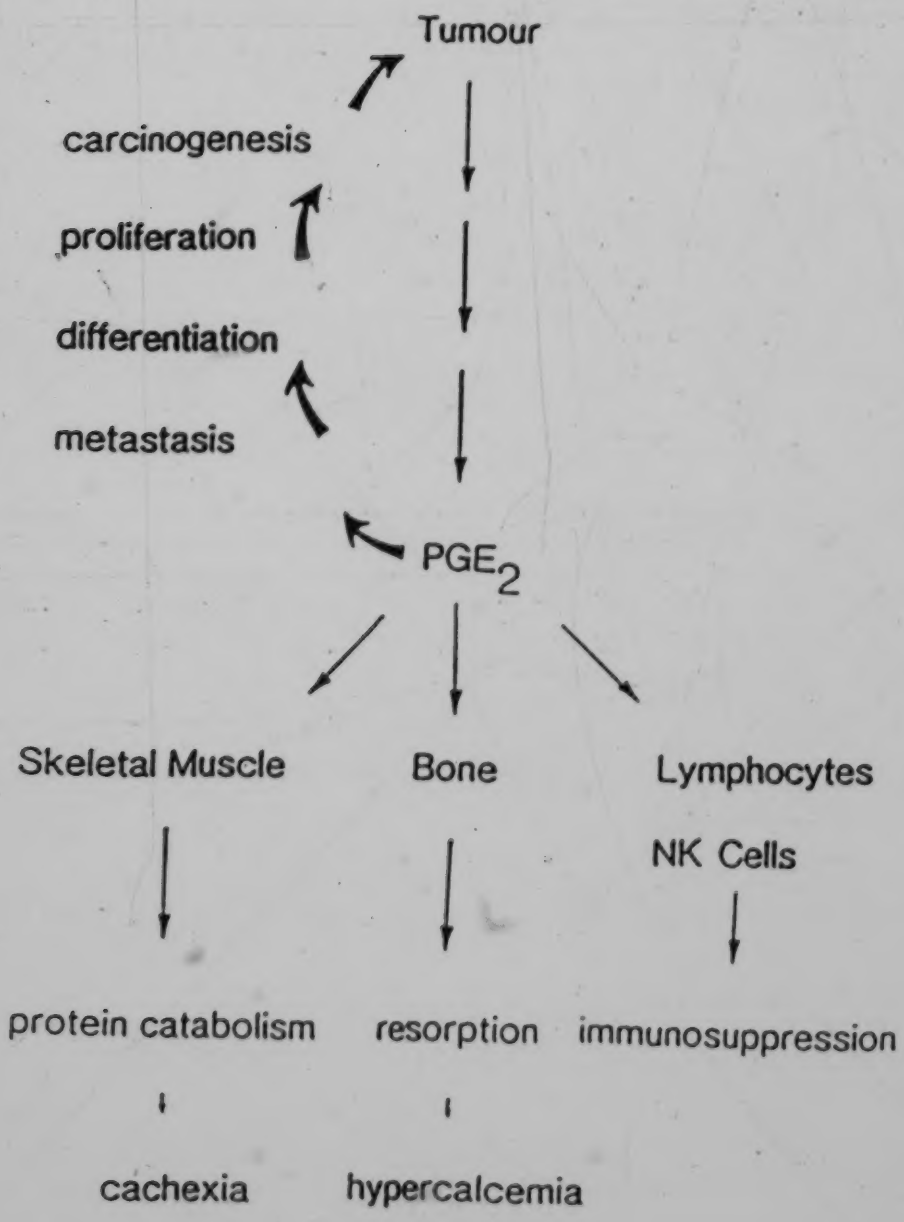
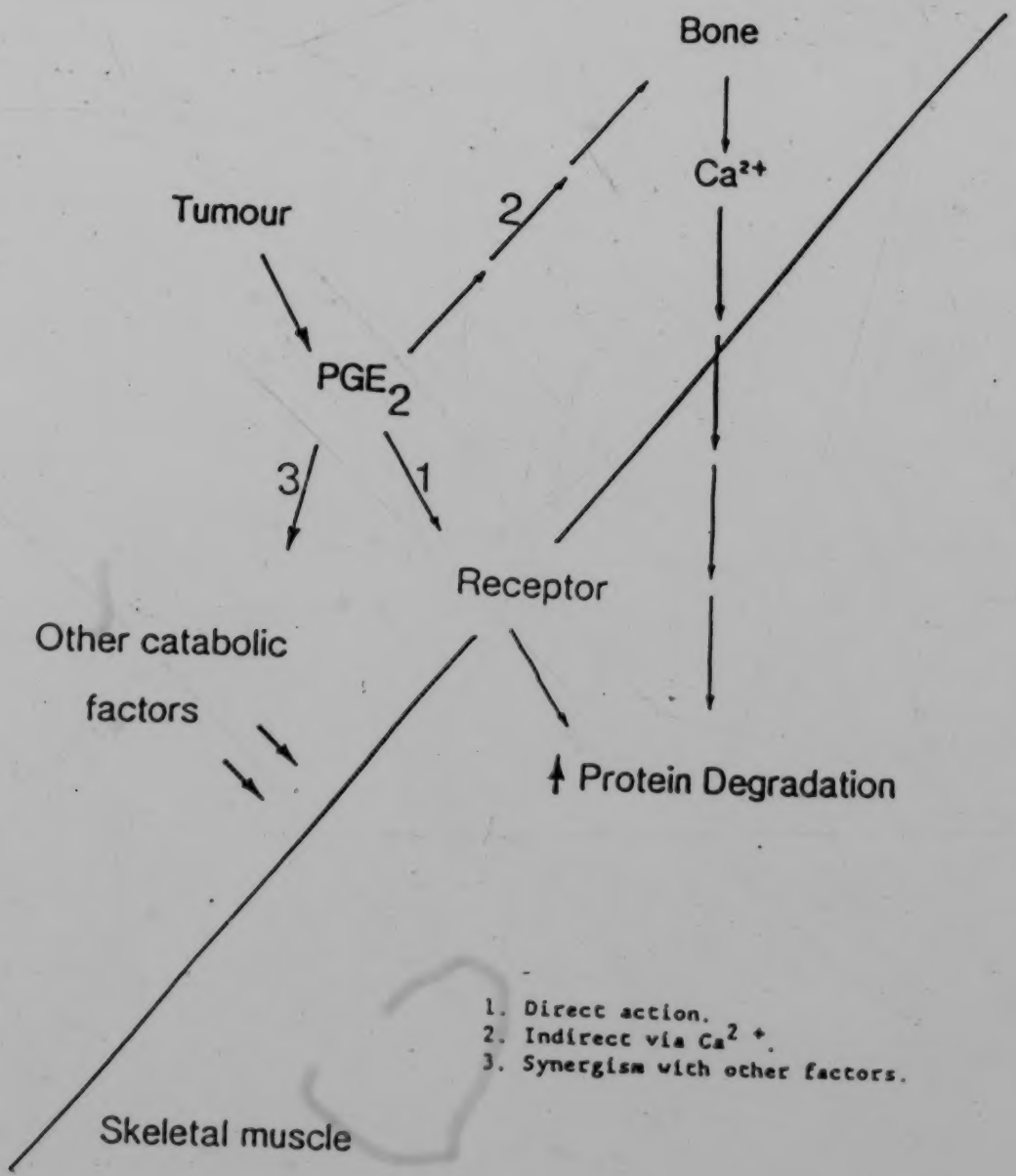


Figure II.3 POSSIBLE MECHANISMS OF PGE_2 ACTION



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APPENDIX

EFFECTS OF NAPROXEN TREATMENT ON BODY TEMPERATURE OF RATS INJECTED WITH ENDOTOXIN.

Table II.10 Effects of naproxen on body temperature of rats 6 hs after injection of E. Coli endotoxin.

	Body Temperature
Control (0.5ml saline):	36.6[a]
Naproxen + endotoxin:	36.8[a]
Naproxen + control:	36.8[a]
Control + endotoxin:	37.7[b]

n= 9 rats per treatment. $P<0.05$, Male Sprague Dawley rats of approximately 120g body weight were used. The dose of naproxen was 11mg/kg Body weight/day which was administered in the drinking water 2 days before the administration of endotoxin. The dose of endotoxin was 100 μg/kg, administered intraperitoneally.